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INVERTEBRATE CONTROL AGENTS - EFFICACY TEST METHODS

VOLUME IX

BACULOVIRUSES AND ENTOMOGENOUS BACTERIA

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

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The purpose of this report is to provide guidance for conducting tests to determine the efficacy of entomogenous bacteria and baculoviruses. The methods described herein are not to be considered to be exclusive of other methods. The entomogenous bacteria developed for pest control are the spore-formers. The baculoviruses are the nuclear polyhedrosis and granulosis viruses. For additional information see publications listed in the General Reference Section of this report.

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VOLUME IX

BACULOVIRUSES AND ENTOMOGENOUS BACTERIA

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BACULOVIRUSES AND ENTOMOGENOUS BACTERIA

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INTRODUCTION

Social and political concerns for the quality of man's environment as well as increasing problems of pest resistance to chemical pesticides have stimulated the research and development of biological agents as alternatives to chemicals for pest control. Insect pathogens, especially the entomogenous bacteria and baculoviruses, ideally fit into this approach.

The purpose of this report is to provide guidance for conducting tests to determine the efficacy of entomogenous bacteria and baculoviruses.

The methods described herein are not to be considered exclusive of other methods. Methodologies and recommended practices for evaluating microbial agents for pest control are few in number compared to those available for testing chemical pesticides. Different situations may require special methods, and new approaches will have to be developed. Every effort was made to keep the suggested test methods broad to cover a wide range of conditions. More detailed information may be obtained by referring to the literature citations in the guidelines.

The entomogenous bacteria developed for pest control are the sporeformers. Non-sporeformers may offer potential but have not been adequately studied as pest control agents. The baculoviruses are the nuclear polyhedrosis and granulosis viruses. They are the most studied group of insect viruses and several have been used for pest control in recent years. For additional information see publications listed in the General Reference section.

Entomogenous bacteria and baculoviruses possess many unique features. Special considerations and, in some cases, modifications of the methods used to test chemical insecticides for efficacy, may be required. Factors to be considered are many:

1. The pathogens must be ingested to be effective. Therefore, consideration must be given to the reproductive activity and feeding behavior of the insect. Method and timing of application can be very critical.
2. All insect pathogens have an incubation period, and observable effects may be delayed for several days. Evaluation intervals should be adjusted accordingly.
3. Insect pathogens may be adversely affected by ultraviolet radiation, and protective measures are often necessary.
4. Biological activity of stored microbial agents can be reduced by exposure to high temperatures, and cool storage conditions should be used.
5. Microbial agents are pH sensitive and the pH of formulations, tank mixes, as well as the substrate to be treated should be monitored. Buffering agents can be used to control problem situations.

6. Treated plants can grow rapidly and dilute field deposits of microbial agents. Repeated applications at close intervals may be necessary to maintain an effective level of control.
7. Anti-bacterial or anti-viral substances produced by target plants may interfere with the effectiveness of a microbial agent.
8. Formulation, method of application and application equipment may influence coverage, persistence and overall performance of a microbial insecticide.
9. Some pathogens applied for insect control may persist in an area for several generations of the target species, or several seasons, and may contribute to long-term suppression of a pest.

Much of the interest in utilizing baculoviruses and entomogenous bacteria for pest control has been stimulated by research results which have shown that (1) they are host-selective, environmentally nondisruptive control agents well-suited for use in integrated control and other pest management programs; and (2) they have not been shown to be hazardous to man, other mammals and plant life. They are very adaptable, and may be employed:

1. As one would use a chemical insecticide;
2. As replacement for a chemical insecticide where the latter is no longer effective because of insect resistance;
3. To provide relief in situations where the use of an otherwise effective pesticide is restricted;
4. As a substitute for parasites and predators where they have been reduced by chemical pesticide;
5. To complement naturally-occurring parasites, predators, and pathogens for pest control; and
6. As an aid in controlling several pest species simultaneously when used in a mixture of pesticides.

The most common approach to the dissemination of microbial agents has been to employ the same equipment used for the application of chemical insecticides. Other ways demonstrated for the application of microbial agents include (1) seeding a microbial pathogen into the target population; (2) the use of traps to capture, externally contaminate with a pathogen and then release wild insects; (3) rear and release parasites which have been externally contaminated with a pathogen.

In the United States, all pesticides are registered under FIFRA. The requirements for the registration of pesticides of biological origin are essentially the same as those prescribed for chemical products. Thus, for registration purposes, there must be clear and convincing evidence that the product to be marketed is effective as stated on the label, and safe when so used. The registration of microbial agents must follow a carefully planned approach which can be summarized as follows: (1) identification of the insect pathogen by various criteria including morphology, growth requirements, stability, bioassay and infectious process; (2) assessment of effects on vertebrate and invertebrate non-target organisms, including acute, subacute, and long-term toxicological studies; (3) small-scale field tests to gather data on efficacy, as well as to monitor effects on the environment; and (4) large-scale field tests to determine efficacy and usefulness under commercial conditions.

GENERAL METHODS

The early stages of development of a microbial agent include the isolation, characterization and identification of the pathogen and the application of the Koch's postulates. The safety of the pathogen for non-target organisms, especially humans, is determined (see "Guidance for Safety Testing of Baculoviruses", published by the U.S.E.P.A., Summers et al, 1975). The requirements for safety testing are influenced by the proposed uses of the pathogens. In some situations a temporary exemption from the requirement of a tolerance must be obtained with an experimental use permit for field testing. In other situations where it has been demonstrated that the use of an insect pathogen does not alter the diversity of insect pathogens naturally present in the environment, and does not increase the residue above that which occurs naturally, the negligible residue concept may be applied.

Laboratory Studies

Bioassay: -- Bioassays are necessary in the initial stages of microbial insecticide development for studying the comparative pathogenicity of various pathogen isolates either in crude or formulated form, and for studying the susceptibility of various target and non-target species or populations. Also, formulated products may be standardized using bioassays.

Bioassays in insects are used to establish the virulence, pathogenicity, and infectivity of a candidate pathogen. This will aid in selecting initial trial dosages for field tests. Bioassay can be employed also to determine compatibility of a candidate pathogen and other agent, chemical, etc.; the effect of application methods and equipment on the activity of a pathogen; and plant coverage, specifically if deposition of the pathogen was in the target areas of the plant. It can also be utilized to study residual persistence of the applied pathogen.

Dosage Selection: -- The selection of an appropriate dosage of a pathogen for field studies depends on laboratory data accumulated against the specific pest. Results obtained from bioassay followed by greenhouse trials are useful for rate determinations. It is important to establish the lowest level at which initial control is first detected and maximum level where additional quantities will not result in substantial increase in control. A standard insecticide treatment commonly used in actual control, untreated checks, and where practical, a diluent control should be included for comparison with experimental materials.

Insect-Rearing and Propagation of Pathogens: -- The establishment and maintenance of laboratory insect cultures is useful and necessary to provide the test animals for bioassay studies, and as a means of propagating the pathogens to be tested. Methods for establishing and maintaining insect cultures are many and varied, and are described in the literature for many pest species (see General Reference section).

In addition to using the living whole organism several other methods have been suggested for propagation of insect pathogens. These include (1) embryonated eggs or cultures of organs, tissue, dispersed cells, or established cell lines; (2) fermentation media; (3) completely defined chemical substrates; and (4) the use of nonhomologous hosts, produced by fermentation. The available systems for mass production of microbial insecticides are categorized and described in the literature (see General Reference section).

Small-Scale Field Tests

Small plot field testing to support registration is usually begun after investigation of the mode of action, growth characteristics *in vivo* and *in vitro*, and formulation development in the laboratory. Testing during these early stages is adaptable for early screening and subsequent performance evaluations. Small plots facilitate thorough and uniform coverage of the host substrate and permit a maximum number of observations. Such plots facilitate control over variables which may influence efficacy under large scale tests or actual use conditions. Also, they minimize the quantity of experimental material required and the crop acreage necessary for testing. Data on optimal rates, formulations, damage prevention, phytotoxicity, compatibility with adjuvant and behavior of infected insects can be obtained in the small-scale field tests.

Site Selection: -- The general area selected should have a previous history of infestations by the target pests. Uniform population densities are highly desirable. Pest populations should be increasing at the time the tests are initiated, or expected to appear in sufficient numbers to provide measurable differences between treatments. Under certain conditions it may be advantageous to artificially infest a selected number of plants within each plot to ensure uniform distribution of the pest. The test site should be sufficiently isolated (by noncrop areas or untreated crop areas) to reduce the hazard of pesticide drift from treatment of other crops.

Test sites selected should have an even uniform stand of the host plant or a uniform mixture of host plants. For agronomic crops, all tests should be established on commercially grown varieties within the selected test site. The variety selected should be susceptible to feeding by the target pest species. The soil type should be uniform, and prepared with methods consistent with growing the crop commercially. The crop should be planted, grown, and maintained in accordance with accepted local agronomical practices.

Climatic conditions, cropping practices, composition of the ecosystem and other factors often result in a wide variation of target insect population levels. Thus, tests should be conducted in as many locations as possible to provide reliable and applicable information. When possible, test locations should cover the target pest-crop range.

Plot Size: -- Plot size will differ with specific target pests and crops, and will be discussed under the individual pests. Plots should be sufficiently large, or protected by buffer space, to prevent drift of materials that are applied to adjacent plots. They should also be large enough that removal of pest species or plant parts during data collection will not interfere with the overall pest population density or normal development and maturation of the crops. Minimum plot length and width will in part be dictated by the application equipment used. Small plots (0.025 ha) may be used with hand-operated applicators whereas larger plots are required with larger equipment.

Experimental Design and Data Analysis: -- Selection of the experimental design will vary somewhat with pest species, crop and individual preference. Randomized complete block is the most commonly used experimental design for small plot efficacy evaluations. Other designs, i.e., Latin Square, split plot and split block are also applicable and in certain instances it may be preferred. A minimum of 3 replicas is suggested. When target pest density, plant age, plant density, plant vigor and soil type are not uniform, more than 3 replications may be needed. A chemical insecticide may be used as a standard serves as a reference point only. Efficacy less than that of the insecticide standard may not preclude the usefulness of a microbial agent as a pest management tool. Treatment effects should be compared to the untreated checks and where possible, diluent controls.

Initially candidate microbial agents should be applied as a single component rather than in combination with insecticides and fungicides. If combination treatments are used the biological compatibility of the additive with the microbial agent must be determined previously. Compatibility of other additives, adjuvants, carriers, mixes, etc., should also be determined. The activity of the mixture should be checked by bioassay.

The number of field trials conducted with each microbial agent must be sufficient to allow accumulation of data on: (1) optimum dosage, (2) proper timing, (3) treatment intervals, (4) performance at different target pest densities and stages, (5) effects on the various cultivars of the host plant, (6) effects on nontarget species, (7) effects complimentary or antagonistic to naturally occurring biological control agents, (8) influence of application on existing titer of the microbial agent in the environment, (9) performance under different climatic conditions, (10) persistence in the test area, and (11) compatibility with all application systems which may be used to apply the microbial agent.

Due to the relative host specificity of microbial agents, it may be necessary to implement control measures for nontarget pest species during efficacy evaluations. Whenever possible the additional control measures should be nonchemical in nature. When a chemical pesticide is the only alternative, it should be one with a minimum of effect against the target pest species and its naturally occurring biological control complex.

An analysis of variance and multiple range test or other appropriate statistical analyses are employed where necessary to determine the statistical reliability of differences between treatments. Treatment means presented alone should be accompanied by the standard deviation or standard error.

Application and Equipment: -- Candidate microbial agents are applied with equipment and methods known to provide adequate coverage of the plant parts requiring protection. In small plots, knapsack, high clearance, tractor-mounted sprayers, mist blowers and other suitable equipment may be used for liquid applications. The addition of wetting and sticking agents to a candidate microbial agent preparation may be desirable. The volume, pressure and flow rate of the application equipment as well as the number and arrangement of the spray nozzles will vary with the insect and crop under test. Dusts and granular preparations are usually applied with hand dusters and granular applicators. Application equipment should always be accurately calibrated before applying the materials to be tested.

It is desirable to express the dosage in terms of potency or activity e.g., International Units (IU) for *Bacillus thuringiensis.* Potency of each candidate microbial agent formulation should be monitored throughout the test period using appropriate bioassay methods.

Timing of applications and their number will vary depending upon the properties of the candidate microbial agent, crop and target insect. Meteorological conditions should be recorded during application periods. Information relative to plant coverage and persistence of candidate microbial agent at the target site should be determined (see Exhibit 1).

Equipment should be thoroughly cleaned before and after use. When 2 or more rates of the same microbial agent are to be applied, begin applications with the lowest rate in order to maintain the integrity of the desired dosage.

Sampling Techniques: -- Scientifically valid standardized procedures should be employed for assessing the efficacy of a candidate microbial agent. Methods will vary with crop management procedures, candidate microbial agent and the target species, and will be discussed under each pest or pest commodity in the sections following. In general, criteria to be used include pest population densities and damage estimates before and after treatments as well as yield and/or marketability of the crop at harvest.

Large-Scale Field Tests

Field tests should be conducted using application techniques commonly employed for control of the particular target pest on the crop. A sufficient number of trials should be conducted to cover the host range and geographical distribution of the pest. Testing at this level provides data to indicate efficacy of a candidate microbial agent under operational conditions.

Site Selection: -- Large field tests are conducted at sites in which host-pest conditions are representative of the areas for which the product registration is desired. Where applicable, the tests include the host varieties, host plant ages, cultural practices, pest populations and weather conditions likely to be encountered in actual field operation.

Plot Size and Design. -- Plots must be large enough to permit utilization of commercial equipment and practices. Optimal plot size may vary with candidate microbial agent and commodity being tested. Plots should be replicated; however, if this is not possible, a sufficient number of subsamples must be taken within the treated and untreated areas to provide a reliable measure of effectiveness.

Dosage Selection: -- Rates utilized in large-scale field testing may consist of a range of dosages including the minimum effective rate determined in small plot experiments. Comparison with standard control practices is useful.

Application and Equipment: -- The method of application used must provide adequate coverage of the plant surfaces. High or low-volume (9.38 - 93.81/ha) ground or aerial applications with conventional low-volume systems may be employed. Test materials may be combined with other components of the typical spray program if they are known to be compatible. (See Small-Scale Field Tests —— Application and Equipment, and Exhibit 1.)

Sampling Techniques: -- Sampling techniques may differ with the pest and crop under study and are discussed in the specific commodity or pest sections that follow. Comparisons of yield data including quality and marketability of the crop treated with a candidate microbial agent, standard treatment materials and untreated controls are made. Supportive statements from the investigator in testimony of the degree of control of the candidate materials may be useful.

Reporting Microbial Agent Test Results

Information on the following should be provided as completely as possible in reporting the results of efficacy tests. However, it is recognized that all information listed below may not be available or needed for every situation.

- Name and address of investigator
- Objectives and purpose of study (crop and target species)
- Product used

    formulation
    lot number
    storage conditions (temperature)
    potency

- Cropping practices

  variety and planting date
  plant density and spacing
  other agronomic practices (irrigation, cultivation, fertilization, other pesticides applied)

- Location of study (longitude and latitude, elevation, exposure, orientation, soil type and analysis)

- Experimental design

  plot size
  number of replicates
  sampling procedure
  statistical methods followed

- Application

  methods
  type of equipment (nozzles, type, number, arrangement, direction, pressure)
  materials applied
    - treatment dates
    - dosage per hectare
    - volume of application (ground or air speed)
    - tank mix (pH, water quality)
    - coverage (actual particles/unit area, droplet size and density, volume emitted vs. volume deposited)

- Timing of application

  stage of crops
  density and stage of target species
  time of application (day, hour)
  climatic conditions (temperature, relative humidity, cloud cover, wind speed and direction, precipitation, crop wet or dry)

- Assessment —— pre- and post-treatment

  sample dates
  crop data
    - stage of growth and development
    - parts examined
    - damage
    - phytotoxicity
    - yields
    - quality

target species data
  - population densities
  - stages present

data on associated species (pests, parasites, predators, other)
  - identification
  - where sampled
  - population densities
  - stages present
  - distribution

data on microbial insecticide
  - residue on crop
  - incidence/persistence (in host, in other species)

- Determining effectiveness and usefulness

degree of protection provided
cost of applications
  - material
  - equipment
  - time

return
  - value of crop (e.g., recreation value, aesthetic value, non-timber value)
  - production

practicality of methods

- Other comments

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ANNUAL ROW CROPS

Methods of efficacy evaluation of baculoviruses and bacteria on annual row crops may not differ appreciably from that for chemical insecticides. The relatively short time that the host plant is present, and the relative high crop value per production unit, particularly for such crops as vegetables and tobacco, may necessitate that a strict regimen be followed to provide economic suppression of the pest population. To gain producer acceptance and use, results with microbial agents alone or as part of the overall pest management program must provide good crop protection.

In general, the lepidopterous pests of annual row crops are multivoltine and feed on a variety of hosts. The crop to be protected may be only one in a succession of hosts that is attacked during the seasonal activity of the pest. As a result, protection against a specific pest may be required for only 1 or 2 generations. With the short-term characteristics of the crop and pest problem, multi-cropping and crops rotation, long-term evaluation of microbial agents on most annual crops may be difficult. In this section emphasis is placed on short-term effects of microbial agents on pest population levels and crop protection. The long-term effects of microbial agents should not be neglected. Data should be collected on the total seasonal effects of the microbial agent on the target pest and non-target species.

The following test methods for annual row crops apply to dilute and concentrate sprays, dusts, granules and baits. Only exceptions to and variations from the procedures described in the General Methods are presented. In addition, an attempt was made to standardize methods with those described in *Analysis of Pesticide Problems, Invertebrate Control Agents Efficacy Test Methods: Volume II —— Foliar Treatments II.* (AIBS Report to EPA. EPA-540110-77-001. 1977.)

FIBER CROPS

Cotton, *Gossypium hirsutum*

Insect pests are generally present on cotton throughout the producing areas of the United States in sufficient numbers to affect yields seriously unless control measures are applied. More chemical pesticides are used on cotton than on any other crop. Thus the development and eventual widespread use of microbial agents on cotton could contribute considerably to reduction of the chemical pesticide load in the cotton agroecosystem. Cotton insect pests currently amenable to suppression by baculoviruses and bacteria include the boll and square feeders —— bollworm, *Heliothis zea* (Boddie), tobacco budworm, *Heliothis virescens* (Fabricius), and pink bollworm, *Pectinophora gossypiella* (Saunders); and the foliage feeders —— armyworms, *Spodoptera* spp., cabbage looper, *Trichoplusia ni* (Hubner), and cotton leaf worm, *Alabama agrillacea* (Hubner).

Boll and Square Feeders: Bollworm, *Heliothis zea* and Tobacco Budworm, *Heliothis virescens*

Plot Size: -- Plot size may vary considerably depending upon uniformity of infestation, population density, application equipment used and personal preference. In small plot tests 0.04 ha is generally accepted as standard. Smaller plots, 8-12 rows by 15-30 m, have been used successfully by a number of investigators. Large plots are usually 24-96 rows by 150 m for ground equipment and 3 or more 12 m swaths by 300 m for aerial application. In the latter stages of product development large plots may cover entire fields.

Application Equipment: -- A variety of equipment has been used for small plot applications, ranging from individually constructed sprayers to commercially produced apparatus. Knapsack or high clearance sprays with a minimum of 2 hollow cone nozzles per row operating at appropriate pressures to deliver 36-124 l per hectare are commonly used. Dusts or granules are usually applied by hand or with small commercial applicators.

Large plot applications may be made with high clearance sprayers (36-62 l/ha) or by air (24-62 l/ha). Most late season applications are by air.

Timing and Frequency of Application: -- The initial application may be made when 6,200-16,400 eggs or 1st stage larvae are present per hectare. Repeated applications at 4-6 day intervals may be required through the fruiting cycle of the plant.

Sampling and Evaluation: -- Weekly egg, larval, damaged square and damaged boll counts made weekly through the treatment period from the center rows of each plot may be helpful in evaluating efficacy. It is suggested that the data be reported in numbers per hectare. Methods of sampling have varied with individual researchers, with whole plant counts on 2-4 m of successive row per plot most commonly used. In addition to damage data, reporting of number of undamaged squares and bolls per hectare may also be useful in evaluating effectiveness.

Yield samples from small plots have been most commonly obtained from the center two or more rows of each plot and reported in kilograms seed cotton per hectare. In large plots, the entire plot or sample area may be mechanically harvested.

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Pink Bollworm, *Pectinophora gossypiella*

Plot Design: -- Field cages, 1.8 x 1.8 x 10.9 m, have been used for efficacy evaluation of microbials against *Pectinophora gossypiella*. It is suggested that the plants in the cages be sprayed with a non-residual insecticide to remove other pest species. 10 pairs of 2-day old *P. gossypiella* adults released in each cage 1 day prior to the 1st application with additional releases at 10 and 15 days provide infestation pressure. Adults from overwintering larvae have provided more reliable infestation than laboratory reared adults.

For open field tests, plot sizes may be comparable to those given for *H. zea* and *H. virescens*.

Application Equipment: -- See *Heliothis zea* and *Heliothis virescens*.

Timing and Frequency of Application: -- In field cage studies the first application may be made 1 day following release of adults with subsequent applications at 5-day intervals until the test is terminated.

In open field tests, applications may be initiated when susceptible squares are present on the plants and adult pink bollworms are detected in the field. This early application would be directed toward the larval generation that develop in squares. Others have initiated application when 850 or more larvae per hectare were present in blooms. Applications may be continued at 4-6 day intervals as long as infestation pressure persists.

Sampling and Evaluation: -- Bloom counts, i.e., normal vs. rosetted, may be useful in evaluating the effect of the treatment, especially on that generation developing during the 1st 6 weeks of the season. An estimation of the total number of blooms and total rosetted blooms in each plot or cage may be determined. When bolls are present, the larval population may be estimated by determining the number of bolls per plot and collecting a minimum of 50 bolls per plot. These bolls may be incubated in suitable containers with paper for pupation sites and the number of larvae exiting the bolls determined. Others have obtained estimates of the number of larvae entering bolls by opening samples of bolls and examining the inside of the carpel for larval mines.

Yield data may be taken as outlined for *Heliothis zea* and *Heliothis virescens*.

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Foliage Feeders: Armyworms, *Spodoptera* spp.; Cabbage Looper, *Trichoplusia ni*; Cotton Leaf Worm, *Alabama argillacea*

Plot Design: -- See also Boll and Sqaure Feeders: *Heliothis zea* and *Heliothis virescens*. With heavy, uniform infestations small plots, 4 rows by 23 m, may be adequate. When small plots are used, a minimum buffer of 4.5 m between blocks and 2.0 m between plots within blocks is recommended.

Application Equipment: -- See Boll and Square Feeders: *Heliothis zea* and *Heliothis virescens*.

Timing and Frequency of Application: -- Multiple applications may not be required with discrete populations. Treatment is recommended when a majority of the larvae are in the 1st or 2nd instars. If multiple applications are necessary, they may be made at 4-6 day intervals.

Sampling and Evaluations: -- Larval counts, defoliation estimates, and yield may prove useful in evaluating treatments. For other foliage feeders, larval counts may be made with sweep net, D-vac, or shake cloth methods. 4 random samples are normally taken from the center of each plot pretreatment, and 5, 10, and 14 days posttreatment with counts converted to numbers per hectare.

Yield data may be taken as outlined for *Heliothis zea* and *Heliothis virescens*.

References

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OIL CROPS

Corn, *Zea mays*

Insect pests of corn that are promising candidates for efficacy evaluations with bacteria and baculoviruses include ear feeders — corn earworm, *Heliothis zea* (Boddie); whorl and ear feeders — corn earworm, *H. zea*, and fall armyworm, *Spodoptera frugiperda* (J. E. Smith); and stalk borers — European corn borer, *Ostrinia nubilalis* (Hubner) and Southwestern corn borer, *Diatraea grandiosella* (Dyar). Procedures for evaluating efficacy of microbial agents on corn were developed on sweet corn. These procedures are also applicable to field corn and are presented in the Vegetable Crops: Sweet Corn section.

Peanuts, *Arachis hypsgaea*

Insect pests of peanuts that may be amenable to control with bacteria and baculoviruses include the foliage feeders —— corn earworm, *Heliothis zea* (Boddie), fall armyworm, *Spodoptera frugiperda* (J. E. Smith), velvet-bean caterpillar, *Anticarsia gemmatalis* (Hubner), rednecked peanutworm, *Stegasta bosquella* (Chambers); and the pod and peg feeder —— lesser corn stalk borer, *Elasmopalpus lignosella* Zeller. Literature on efficacy evaluations of microbials for control of these pests is wanting. The following procedures are based upon those used for these pests on other crops and with chemical insecticides on peanuts.

Foliage Feeders: Corn Earworm, *Heliothis zea*; Fall Armyworm, *Spodoptera frugiperda*; Velvetbean Caterpillar, *Anticarsia gemmatalis*; Rednecked Peanutworm, *Stegasta bosquella*

Plot Design: -- Small plot size averages 4-8 rows by 15-30 m with large plots 24-96 rows by 150 m for L. V. ground application and 45-60 m by 150-300 m for aerial or mistblower application.

Application Equipment: -- The first application should be made when the number of 1st or 2nd stage larvae per row meter approaches 3-4. Larval number may be determined by use of sweep net, shake cloth or D-vac. One application is usually adequate, but should oviposition occur over an extended period, repeated applications at 4-6 day intervals may be required.

Sampling and Evaluation: -- Efficacy evaluations are usually based upon larval numbers, defoliation, or yield. Larval numbers may be determined by sweep net, 20 sweeps with a 0.4 m net across 2 adjacent rows in the center of each plot; by shake cloth, shake the plants at 4 locations per plot over a 1 m cloth; or D-Vacing 12 m of row per plot. Larval counts are usually made 5 and 10 days following the last application.

Defoliation may be determined visually and rated as follows: none, light —— 1-20%, moderate —— 21-40%, heavy —— greater than 41%.

Yield samples should be taken from the center two rows of each plot either manually or with a commercial thresher.

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Peg and Pod Feeders. Lesser Cornstalk Borer, *Elasmopalpus lignosellus*

Plot Design: -- See Foliage Feeders: *Heliothis zea, Spodoptera frugiperda, Anticarsia gemmatalis, Stegasta bosquella.*

Application Equipment: -- See Foliage Feeders: *Heliothis zea, Spodoptera frugiperda, A. gemmatalis, S. bosquella.* Granular applications may be made by hand or with commercial applicators.

For basal directed sprays, a flat fan 80° nozzle should be directed on each side of the row so as to cover the soil and lower leaves. A minimum of 180 l total volume per hectare is recommended.

Granular or bait applications should be made when the foliage is dry in a 0.45-0.51 m band over the row.

Timing and Frequency of Application: -- Foliar applications should be timed at peak moth flight with granular applications made 20-30 days after planting.

Sampling and Evaluation: -- At 7, 14, 21 and 28 days posttreatment 10-20 plants at 4 locations in the center of each plot are examined for live borers and damage. Number of larvae, plants infested, pegs damaged and nuts damaged are recorded.

The center two rows of each plot should be harvested with yield and grade of cured peanuts recorded.

References

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Soybean, *Glycine max*

Insect pests may be present in soybean in sufficient numbers throughout the United States to seriously affect yield unless control measures are applied. Of these the pod feeder, *Heliothis zea* (Boddie) and the foliage feeders —— soybean looper, *Psuedoplusia includens* (Clemens); green cloverworm, *Plathypena scabra* (Fabricius); velvetbean caterpillar, *Anticarsia gemmatalis* (Hubner); beet armyworm, *Spodoptera exigua* (Hubner); cabbage looper, *Trichoplusia ni* (Hubner); and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) are the most likely candidates for control with bacteria and baculoviruses.

Pod Feeders, Corn Earworm, *Heliothis zea*

Plot Design: -- Plots 4-8 rows by 15-30 m are adequate for small scale evaluations. Suggested plot size for large scale evaluations is 24-96 rows by 150 m for ground equipment and 36 by 300 m for aerial application.

Application Equipment: -- Knapsack, high clearance or other L. V. small plot equipment may be used. Sprayers should be equipped with 2 hollow cone nozzles per row operating at appropriate pressures and ground speed to deliver 36-60 l per hectare. Dusts may be applied by hand or with commercial dusters.

Timing and Frequency of Application: -- Treatments are usually made when 4 or more 1st or 2nd stage larvae are present per row meter. Additional applications may not be required.

Sampling and Evaluation: -- At 5 and 10 days posttreatment larval counts are made by shaking or beating the plants over a ground cloth, 3 m in length. A minimum of 4 points should be sampled per plot. The number of live larvae are recorded and converted to numbers per hectare.

Yield samples may be taken from the center rows of each plot.

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Foliage Feeders: Soybean Looper, *Pseudoplusia includens*; Green Cloverworm, *Plathypena scabra*; Velvetbean Caterpillar, *Anticarsia gemmatalis*; Beet Armyworm, *Spodoptera exigua*; Cabbage Looper, *Trichoplusia ni*; Fall Armyworm, *Spodoptera frugiperda*

Plot Design: -- See Pod Feeders: *Heliothis zea*.

Application Equipment: -- See Pod Feeders: *Heliothis zea*.

Timing and Frequency of Application: -- The first treatment is recommended when 50-65,000 1st or 2nd stage larvae are present per hectare. Applications are repeated at 4-6 day intervals on an as-needed basis.

Sampling and Evaluation: -- See Pod Feeders: *Heliothis zea*.

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Sunflower, *Helianthus annus*

The sunflower moth, *Homoeosoma electellum* (Hulst) is considered the major insect pest of sunflower. The larvae of this insect feed on the developing seeds in the seed head. The corn earworm, *Heliothis zea* (Boddie), and tobacco budworm, *Heliothis virescens* (Fabricius), may also attack seed heads of sunflower.

Seed Head Feeders: Sunflower Moth, *Homoeosoma electellum*; Corn Earworm, *Heliothis zea*; Tobacco Budworm, *Heliothis virescens*

Plot Design: -- Small scale evaluations may be conducted with plots 4-8 rows by 15-30 m. Large plots range from 0.4-4.0 hectares with a 7.5 m buffer area between plots.

Application and Equipment: -- Most applications have been made with a high clearance sprayer or by air. When a high clearance sprayer is used 2 flat fan nozzles per row are directed on the face of the seed head. Volumes from 120-240 l per hectare are suggested.

Timing and Frequency of Application: -- Treatments should be begun when 20% of the heads are in the flower stage. Repeated applications at 4-6 day intervals may be required as dictated by infestation counts.

Sampling and Evaluation: -- At weekly intervals during the treatment period and through 21 days following the last application, 10-25 seed heads should be selected from each plot and examined. The number of heads infested, damaged seed per head and seed yields per head are recorded.

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Sugar Crops

Sugarbeets, *Beta vulgaris* and sugarcane, *Saccharum* spp. are the major sugar producing crops in the United States. Lepidopterous pests of these crops amenable to control with bacteria and baculoviruses include the beet armyworm, *Spodoptera exigua* (Hubner), and fall armyworm, *Spodoptera frugiperda* (J. E. Smith), on sugarbeets and the sugarcane borer, *Diatraea saccharalis* (Fabricius) on sugarcane.

Sugarbeets: *Beta vulgaris*

Foliage Feeders: Beet Armyworm, *Spodoptera exigua*; Fall Armyworm, *Spodoptera frugiperda*

Plot design: -- Minimum plot size is 2 rows by 15 m. An untreated buffer row between plots and 1.5-1.8 m buffer area between blocks is recommended.

Large-scale plots with ground equipment may be 4-6 rows by 30 m and 36 m by 180 m for aerial application.

Application Equipment: -- Small plot applications may be made with knapsack, tractor-mounted, or other similar types of equipment. Fan-type nozzles are usually selected and operated at appropriate pressures to provide 95-151 l per hectare.

Large-scale applications are made with commercial equipment. For ground equipment, the above volume per hectare is suggested. Volume for aerial application usually ranges from 19-38 l per hectare.

Timing and Frequency of Application: -- Treatment is suggested when 50-65,000 1st or 2nd stage larvae are present per acre. Repeated applications should be at 5-7 day intervals on an as-needed basis.

Sampling and Evaluation: -- Efficacy evaluations may be based on larval counts. At 5, 10 and 14 days posttreatment, 5-10 plants are selected at random from 2 or more rows per plot with the number of larvae counted. A visual estimate of damage may be made.

References

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Sugarcane: *Saccharum* spp.

Stalk Borers: Sugarcane Borer, *Diatraea saccharalis*

Plot Design: -- Small-scale evaluations may be conducted in plots 3 rows by 7 m. Large-scale plots for ground equipment average 4-6 rows by 30 m long. Aerial applications should be on plots a minimum of 36 m wide by 180 m in length.

Application: -- Small plot spray applications may be made with knapsack, high clearance or other adaptable sprayers. Fan-type nozzles are suggested and should be operated at appropriate pressures to provide 95-151 l per hectare.

Granules may be applied by hand or with commercial applicators.

Timing and Frequency of Application: -- Treatments should begin after 2nd generation larvae hatch, but before the young larvae bore into the stalk. A minimum of 5% of the stalks should be infested.

Repeated applications are made at bi-weekly intervals.

Sampling and Evaluation: -- At weekly intervals during, and up to two weeks following the treatment period, 25-50 stalks are selected at random in each plot. The number of stalks with leaf sheath feeding and number of live and dead larvae are recorded.

At harvest, 25-50 stalks are selected from each plot and the number of joints tunneled determined.

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Tobacco

Tobacco, *Nicotiana tobacum*, is a relatively localized, restricted acreage crop with a high cash value per production unit. The crop is susceptible to damage by a number of lepidopterous pests. These include bud and seed feeders —— tobacco budworm, *Heliothis virescens* (Fabricius), and corn earworm, *Heliothis zea* (Boddie); and foliage feeders —— tobacco hornworm, *Manduca sexta* (Linneaus), tomato hornworm, *Manduca quinquemaculata* (Haworth), cabbage looper, *Trichoplusia ni*, and a variety of cutworms.

Bud and Seed Pod Feeders: Tobacco Budworm, *Heliothis virescens*; Corn Earworm, *Heliothis zea*

Plot Design: -- For small scale field testing, plots may range from 2-4 rows by 10-15 m with a two-row buffer between plots and 3-6 m buffer between blocks. Large plots average 2-4 times the size of small plots.

Application Equipment: Knapsack, high clearance, and other small plot sprayers may be used in small-scale testing. The sprayers are usually equipped with 1 full or disc cone nozzle per row positioned 0.3-0.45 m above the plant. Recommended volume ranges from 370-560 l per hectare.

Dust or granules may be applied by hand or with commercial applicators.

Timing and Frequency of Application: -- Treatments may be begun when 5-10% of the plants are infested with 1st and 2nd stage larvae. Repeated applications may be made at 4-6 day intervals as long as the infestation persists or until flower buds appear. Where infestations are irregular or nonuniform artificial infestation of selected plants in each plot with newly hatched larvae from laboratory colonies has proven useful.

Sampling and Evaluation: -- Larval counts and damage estimates are helpful in evaluating efficacy. At 3, 6 and 10 days posttreatment, 25-30 plants are examined in the center of each plot. With more than one application, larval counts may be made at 3-day intervals and 3, 6 and 10 days following the last application. The number of larvae and percent of plants infested are recorded. At the final examination the number of leaves consumed per larva is visually estimated to the nearest 0.1 leaf. When damage ratings are used the parameters and limit of each rating category should be defined.

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Foliage Feeders: Tobacco Hornworm, *Manduca sexta*; Tomato Hornworm, *Manduca quinquemaculata*; Cabbage Looper, *Trichoplusia ni*

Plot Design: -- See Bud and Seed Pod Feeders: *Heliothis virescens* and *Heliothis zea.*

Application Equipment: -- See Bud and Seed Pod Feeders: *Heliothis virescens* and *Heliothis zea.*

Timing and Frequency of Application: -- Applications may begin when 5-10% of the plants are infested with 3rd and 4th stage larvae. One application is usually sufficient for hornworm control. Multiple applications may be required for cabbage looper and made at 5-7 day intervals until larval density drops below 5% infested plants.

Sampling and Evaluation: -- See Bud and Seed Pod Feeders: *Heliothis virescens* and *Heliothis zea.*

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Stalk and Foliage Feeders: Dark-side Cutworm, *Euxoa messoria* (Harris)

A number of cutworm species are reported to attack transplanted tobacco. Although the procedures outlined below are for *Euxoa messoria*, they are applicable to other species. Further, these procedures may also be applicable for efficacy evaluations of bacteria and baculoviruses against cutworms on other annual row crops.

Plot Design: See Bud and Seed Pod Feeders: *Heliothis virescens* and *Heliothis zea*.

Application Equipment: -- Due to the mode of action of bacteria and baculoviruses and the habits of cutworms, only granular baits are suggested. These may be applied by hand or with commercial applicators.

Timing and Frequency of Application: -- Treatments are made preplant over the soil surface of the bed or postplant over the plants. Repeated applications are not advised.

Sampling and Evaluation: -- All plants are examined in the center 2 rows of each plot for cutworm damage and larval populations. Counts should be made at weekly intervals until larval development is completed. Damage is usually recorded as percent damaged plants per plot.

Other: -- To prevent interplot cutworm migration, aluminum barrier strips 20.3 cm may be embedded in the soil to a depth of 7.6 m encircling each plot.

References

Cheng, H. H. 1971. Field studies on the chemical control of the dark-sided cutworm (Lepidoptera: Noctuidae) on tobacco in Ontario, with particular reference to Dursban. *Can. Entomol.* 103: 649-653.

Cheng, H. H. 1973. Laboratory and field tests with *Bacillus thuringiensis* against dark-sided cutworm, *Euxoa messeria* (Lepidoptera: Noctuidae) on tobacco. *Can. Entomol.* 105: 941-145.

Cheng, H. H. 1973. Further field evaluation of insecticides for control of the dark-sided cutworm (Lepidoptera: Noctuidae) on tobacco in Ontario. *Can. Entomol.* 105: 1351-1357.

Vegetable Crops

Crops which will be examined in this section include crucifers, lettuce, cucurbits, potatoes, beans, peas, peppers, snap beans, lima beans, southern peas, sweet corn, and tomatoes. Insect pests are confined to the order

Lepidoptera. Often test methods described are not specific for microbial insecticides but are indicative of adequate test procedures for the insect on the specific crop reviewed.

Crucifers

Crops in this group include cabbage, *Brassicae oleracea* var. *capitata*; broccoli, *Brassica oleracea* var. *italica*; cauliflower, *Brassica oleracea* var. *capitata*; Brussels sprouts, *Brassica oleracea* var. *gemmifera*; kale, *Brassica oleracea* var. *ocephala*; collards, *Brassica oleracea* var. *virdis*; turnip, *Brassica campestris* var. *rapa*; mustard, *Brassica juncea* var. *crispifolia*; spinach, *Spinacia oleracea*; and kohlrabi, *Brassica caulorapa*.

Pests in this group include cabbage looper, *Trichoplusia ni* (Hubner); imported cabbage worm, *Pieris rapae* (Linnaeus); diamond back moth, *Plutella xylostella* (Linnaeus); fall armyworm, *Spodoptera frugiperda* (J. E. Smith); beet armyworm, *Spodoptera exigua* (Hubner); garden webworm, *Loxostege rantalis* (Guenee); Hawaiian beet webworm, *Hymenia recurvalis* (Fabricius); and corn earworm, *Heliothis zea* (Boddie).

Cabbage looper is the major pest of the crucifers. The other pests may be sampled and recorded using the same basic techniques used for cabbage loopers.

Plot Design: -- Plot size will vary depending on population uniformity, distribution and density. Randomized complete blocks, with three or more replicates are recommended. Plots may consist of either single or multiple rows, 8-15 m in length. Buffer rows are essential to 1 and 2 row plots and desirable in larger plots.

Aerial applications require larger plots. These should consist of a minimum of 2, and preferably 3, swaths at least 12 m wide. This aids in minimizing the effects of drift and ensures a sufficiently sized central area for collection of representative samples.

Application Equipment: -- Knapsack sprayers, if calibrated carefully, will give satisfactory results. When single nozzles are used the top and sides of each row should be covered.

A wide variety of pressures and rates are used in commercial application. Generally, the type of application equipment is of secondary importance to good coverage. To optimize coverage spraybooms should be equipped with at least 3 nozzles per row and volumes of tank mix ranging from 187-935 l/ha should be applied.

Equipment adaptable and consistent with accepted practices in the given locality should be used for tests in large plots. Tank, boom and nozzles should be thoroughly cleaned before use and when changing treatments. If more than one rate is tested, start test sequence with the lowest rate to lessen chances of cross-contamination from other treatment material.

Timing and Frequency of Application: -- Spraying should begin when an economic population level is present in the plots. Efforts should be made to initiate treatment when insects are in the first instar. Spray intervals should be 5-7 days during the active cycle of the target insect. Observations should be made at 5-7 day intervals to determine degree of initial kill and relative residual activity of the microbial insecticide.

Sampling and Evaluation: -- Larvae should be counted on at least 10-25 plants per plot. Surviving loopers should also be grouped according to size.

Larval counts should be supplemented with ratings of the crop injury. Various ratings can be employed (1-10 scale, 1-6 scale, gradation of damage on edible parts of the plant). Terminology such as medium, severe, etc., should not be used unless clearly explained.

Yield records detailing head weight, marketability, etc., should be taken.

References

Chalfant, R. B., and C. H. Brett. 1965. Cabbage looper and imported cabbageworms: Feeding damage and control on cabbage in western North Carolina. *J. Econ. Entomol.* 58(1): 28-33.

Chalfant, R. B., W. G. Genung, and R. B. Workman. 1973. Control of the cabbage looper in Florida and Georgia. *J. Econ. Entomol.* 66(1): 276-277.

Creighton, C. S., and T. L. McFadden. 1974. Complementary actions of low rates of *Bacillus thuringiensis* and chlordimeform hydrochloride for control of caterpillars on cole crops. *J. Econ. Entomol.* 67(1): 102-104.

Creighton, C. S., and T. L. McFadden. 1975. Cabbage caterpillars: Effects of chlordimeform and *Bacillus thuringiensis* in spray mixtures and the comparative efficacy of several chemical and *B. thuringiensis* formulations. *J. Econ. Entomol.* 68(1): 57-60.

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Greene, G. L., and R. B. Workman. 1971. Cabbage looper control on collards in Florida. *J. Econ. Entomol.* 64(5): 1331-1332.

Hale, R. L., and H. H. Shorey. 1972. Cabbage looper control on cole crops in southern California: Granular insecticides in the soil indicate lack of promise. *J. Econ. Entomol.* 65(6): 1658-1661.

Jacques, R. P. 1973. Tests on microbial and chemical insecticides for control of *Trichoplusia ni* (Lepidoptera: Noctuidae) and *Pieris rapae* (Lepidoptera: Pieridae) on cabbage. *Can. Entomol.* 105: 21-22.

Kouskolekas, C. A., and J. D. Harper. 1973. Control of insect defoliants of collards in Alabama. *J. Econ. Entomol.* 66(5): 1159-1161.

Cabbage Looper, *Trichoplusia ni* (Hubner)

The following methods will also apply to other pests such as imported cabbageworm, armyworm, diamond back moth, and beet armyworm.

Plot Design: -- Small-scale ground applications may consist of single plots one row wide by 8-15 m in length. Buffer rows (1-2) should be included between plots to prevent drift and movement of insects. Commercial ground applications should be sized to handle the common spray applicators normally used by growers. These should be 6-8 beds wide by 15-20 meters in length. Aerial applications require large plots consisting of 2 or preferably 3 swaths at least 12 m wide. This aids in preventing drift and ensures a sufficiently sized central area for collection of representative samples.

Application Equipment: -- See section under crucifers.

Timing and Frequency of Application: -- Spraying should begin when an economic population level is present in the plots. Efforts should be made to initiate treatment when larvae are in the first instar. Spray intervals should be 5-7 days during the active cycle of the target insect. Observations should be made at 5-7 day intervals to determine degree of initial kill and relative residual activity of the microbial insecticide.

Sampling and Evaluation: -- A larval count on at least 10 plants per plot should be made. Care should be taken when examining single row hand plots to keep at least 1.5 m away from the ends of each plot. For large test plots, where application has been made by ground or air, evaluations should be restricted to a well-defined buffer zone within the test area.

To determine effects on yield all mature lettuce heads in the central part of each plot should be graded and marketable heads put in cartons. Convert data to cartons per hectare.

References

McCalley, N. F., and Der-I-Wang. 1972. Field evaluation of insecticides for control of green peach aphid and alfalfa looper on head lettuce. *J. Econ. Entomol.* 65(3): 794-796.

Vail, P. V., C. F. Soo Hoo, R. S. Seary, R. G. Killinen, and W. W. Wolf. 1972. Microbial control of lepidopterous pests of fall lettuce in Arizona and effects of chemical and microbial pesticides on parasitoids. *Env. Entomol.* 1(6): 780-785.

Curcurbits

Crops in this group include cantaloupe, *Cucumis melo* var. *cantalupensis*; cucumber, *Cucumis sativus*; pumpkin, *Cucurbita pepo*; squash, *Cucurbita maxima*; and watermelon, *Citrullus vulgaris*.

Pickleworm, *Diaphania nitidalis* (Stoll) and melonworm, *Diaphania hyalinata* (Linnaeus)

The insects are similar and can be evaluated by essentially the same techniques. However, to date insecticides manufactured from bacteria or baculoviruses have been unsuccessful in controlling this pest complex. Methods described will therefore be general.

The stems, terminal buds and blossoms of muskmelon, cucumber and squash are severely attacked by the pickleworm, *Diaphania nitidalis* (Stoll). The melonworm, *Diaphania hyalinata* (Linnaeus) feeds extensively on the leaves.

Unless foliage injury is severe, treatments should be begun one week before fruit set and weekly applications should begin thereafter.

Plot Design: -- A randomized complete block design and 3 or more replicates per treatment are suggested.

Plot size will vary depending on density and uniformity of the insect population. Plot sizes which have been used have ranged from approximately 8 meters in length by 1-8 rows wide to commercial size plots 4-16 rows in width extending to field length.

Aerial Application: -- 2 and preferably 3 swaths covering an area approximately 182 m long by 36 m wide is suggested.

Application Equipment: -- Ground applications may be made using knapsack sprayers delivering 200-1,000 l per hectare. Sprayers should be calibrated and spray applied along the top and each side of the row. This can be accomplished with a boom having 3 nozzles suitably positioned or a single nozzle. With single nozzle equipment, 3 separate trips down each row will be required to provide thorough coverage of the plants.

Commercial-scale applications should be made according to the accepted spray practices normally used on the crop and area in which the trial is being conducted. Aerial application should use 19-94 l/ha using commercially acceptable nozzle and boom arrangements.

Timing and Frequency of Application: -- Application should begin before fruit set and at weekly intervals thereafter.

Sampling and Evaluation: -- Because microbial agents are slower-acting, evaluations should not be made sooner than 5 days following the final spray. This will ensure that the pathogen has sufficient time to be ingested and kill the insect pest.

Counts should be made of 10-25 fruit or more per plot. The number infested should be recorded and, if possible, the actual worm counts. Frequent checks are necessary since damaged fruit rot and disappear rapidly.

A thorough record should be made of infested stems, terminal buds or blossoms per 10-25 plants, or per plot in the case of the pickleworm. Check foliage of 10-25 plants for melonworm.

Take yields. Count damaged and undamaged fruit; record weight of marketable fruit and average weight.

References

Canerday, T. D. 1967. Control of pickleworm on cucurbits. *J. Econ. Entomol.* 60(6): 1705-1708.

Cabbage Looper, *Trichoplusia ni* (Hubner)

In recent years cabbage looper has become a major pest of cucurbits in Virginia and bordering states. Published references concerning looper control in cucumbers and other cucurbits are unavailable. The experimental techniques and reporting procedures described in the General Methods section and under Crucifers can be used.

Plot Design: -- See section under melonworm and pickleworm.

Application Equipment: -- See section under melonworm and pickleworm.

Timing and Frequency of Application: -- Begin application when loopers first appear on the crop. Apply microbial agent at 5-7 day intervals as required during the active cycle of the pest.

Sampling and Evaluation: -- Efficacy should be evaluated by enumerating looper larvae on 10-25 plants or leaves per plot after each application. Loopers should be divided into size classifications.

References

Hale, R. L., and H. H. Shorey. 1972. Cabbage looper control on crops in southern California: Granular insecticides in the soil indicate lack of promise. *J. Econ. Entomol.* 65(6): 1658-1661.

Tomatoes, *Lycopersicon esculentum*

Tomato fruitworm, *Heliothis zea* (Boddie); tomato hornworm, *Manduca quinquemaculata* (Haworth); western yellow-striped armyworm, *Spodoptera praefica* (Grote); beet armyworm, *Spodoptera exigua* (Hubner); southern armyworm, *Spodoptera eridania* (Cramer); cabbage looper, *Trichoplusia ni* (Hubner); Tomato pinworm, *Keiferia lycopsersicella* (Busck)

The tomato fruitworm, *Heliothis zea*, also known as the corn earworm and cotton bollworm, is the major pest of tomatoes in the United States. When testing bacteria or baculoviruses against this pest, foliar sprays applied during a specific spray schedule at a prescribed interval of 5-7 days should be followed.

Plot Design: -- A randomized complete block design and 3 or more replicates per treatment is suggested.

Single row plots 1.8 m wide and 12 m long have been used, but should be considered minimum. More typical plot sizes are 5.4 m wide (1.8 m rows by 3.6 m long), 4 rows wide by 7.5 m long, and 3 rows wide by 3.6 m long.

Application Equipment: -- Knapsack sprayers and compressed air sprayers (7.6 l) are satisfactory for applying test material to small plots. Volumes per hectare of experimental materials should be in the range of 465-830 l. Uniform coverage should be stressed and the addition of a suitable wetter-sticker is recommended.

Best results in large plots will probably be obtained by using commercial spray practices and equipment.

Timing and Frequency of Application: -- Applications begin at fruit set and at 5-7 day intervals thereafter during the active cycle of the insect.

Sampling and Evaluation: -- Efficacy ratings of the treatments should be based on the percentage of harvested fruit with fruitworm injury. Percentage control is estimated from comparison of the percent injured fruit from treated and untreated test plots.

Because fruitworm injury is often light, 100-200 fruit per plot should be examined. Fruitworm may destroy young tomatoes. If this situation exists, count the total number of infested fruit in 10-25 or more plants per plot and remove the small, infested tomatoes as they are observed. Armyworm damage can be evaluated in a similar manner. Pinworm control is evaluated by recording numbers of marketable and cull fruit lost, yield, mines per leaflet and pupae on mulch beneath plants and at harvest.

Tomato hornworms and cabbage looper control is determined by direct counts of surviving larvae on at least 10 plants in the treated plots. These are compared to the untreated control and percent population reduction determined.

References

See citations following tomatoes: poled (Fresh Market).

Tomato fruitworm, *Heliothis zea* (Boddie) and tomato pinworm, *Keiferia lycopersicella* (Busck)

Plot Design: -- A randomized complete block design and 3 or more replicates per treatment is suggested.

Plots for initial screening can be 3 rows wide by 7.5 m long. For secondary screening larger plots 4-8 rows wide by 15 m long should be used. This will help prevent migration of ovipositing adults into the untreated plots.

Since most commercial ground applicators treat 4 rows per swath, plots under commercial evaluation should be 8 rows wide by 30 m long. The length should be sufficient to harvest a representative sample of fruit.

Application Equipment: -- Hy-Boy type spray equipment with vertical booms on each side of each plant row using 3-5 nozzles (depending on the height of the tomato plant) on each boom would provide good coverage with ground equipment. Properly calibrated aerial equipment may also be used.

For ground equipment the microbial insecticide should be applied in a volume of 930-1860 l/h. Aerial application should be made in the range of 140-184 l/h.

Application should begin when small tomatoes first appear. Preventive treatment schedules should be used and the pathogen applied at 7-day intervals until harvest. When feasible, depending on geographical area and population pressure, more than one time interval should be evaluated.

Sampling and Evaluation: -- Since poled tomatoes are harvested over a period of several weeks, the sampling of fruit for examination should be done when there are enough mature fruit for a representative sample. Tomatoes should be harvested at random over a wide area but at all times within a well-defined buffer zone.

Small screening plots that are 3 rows wide by 7.5 m long should have a fruit selection area one bed wide and 5 ft. from the end of each plot. This method provides a 2-row buffer zone on each side of each plot.

Aerial plots 36 m by 180 m should have a fruit selection area in the middle third of the plot.

A total number of 350-400 marketable tomatoes should be selected at random per treatment regardless of the number of plots.

Each tomato should be examined and the number, species of larvae and damage recorded. Extreme care should be taken to determine if damage is the result of the fruitworm or pinworm.

The total number of tomatoes infested as well as percent tomatoes infested is recorded.

References

Creighton, C. S. 1976. Field tests of insecticidal sprays and baits for control of tomato fruitworm in tomatoes. *J. Ga. Entomol. Soc.* 11(2): 101-105.

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Harding, J. A. 1971. Field comparisons of insecticidal sprays for control of four tomato insects in South Texas. *J. Econ. Entomol.* 64(5): 1302-1304.

Poe, S. L., and P. H. Everett. 1974. Comparison of single and combined insecticides for control of tomato pinworm in Florida. *J. Econ. Entomol.* 67(5): 671-674.

Sweet Corn

Corn Earworm, *Heliothis zea* (Boddie)

Plot Design: -- Randomized complete block design with 3 or more replicates per treatment is suggested.

In the early stages, small-scale applications are useful for demonstrating efficacy. Dusts, sprays, granules and baits may be evaluated. Plots may be 3-5 rows wide by 7.5-15 m long.

Commercial ground applications and larger plots should be done in the final stages of development. Plots should be a minimum of 4 rows wide by 60 m long.

Application Equipment: -- Hand applications of treatment material may be used in preliminary tests. A compressed air or knapsack sprayer is satisfactory. Large-scale field tests should be made with a high-clearance sprayer with 2-4 nozzles per row adjusted to cover only the ear area, particularly the silks.

Spray volumes from 465-930 l/ha should give satisfactory results. Full and uniform coverage should be stressed.

Timing and Frequency of Application: -- Application should coincide with the first appearance of silk on the corn ears. Young larvae hatch from eggs laid in the silk and migrate down into the silk channel between husks. 3-6 applications are required at 2-3 day intervals for adequate control.

Sampling and Evaluation: -- 25-50 ears should be selected at random from the center of each plot. Each ear should be examined and rated as damaged or clean. Data are recorded as "percent ears damaged."

References

See citations following the Sweet Corn section.

Fall Armyworm, *Spodoptera frugiperda* (Smith)

The fall armyworm is often found associated with corn earworm. However, when the fall armyworm infests young sweet corn prior to silk formation, severe damage often occurs. Once the silk stage is reached, plot size and application techniques described for corn earworm apply.

Plot Design: -- See section under corn earworm.

Application Equipment: -- A compressed air or knapsack sprayer is satisfactory for small plots but the test material must be applied from overhead into the whorl. This is necessitated because the armyworm feeds deeply into the whorl of the plant. For larger plots, commercial equipment may be used.

Volumes of spray per hectare are similar to that listed for corn earworm.

Timing and Frequency of Application: -- A 2-3 day application schedule prior to silking or a shorter application period after silking will be required.

Sampling and Evaluation: -- Direct counts of armyworms or grading of armyworm injury in 10-50 corn plants should be done. Leaves should be unrolled and whorls thoroughly examined. Records should be based on fresh damage. Foliar injury ratings may also be useful.

Armyworm infestation in samples of at least 25 ears per plot should be counted. Carefully observe and record the degree of injury into the same classes as used for earworms.

References

See citations following the Sweet Corn section.

European Corn Borer, *Ostrina nubilalis* (Hubner)

The European corn borer is often associated with corn earworm, and generally will be controlled using the same sprays and schedules used for earworms. However, the corn borer deposits its eggs in masses on the undersides of the leaves in clusters up to 50. Young worms bore into various parts of the plant, ears included, and often cause stalks to break and the ear section to come into contact with the ground. Damage to the ears is often extensive rendering it entirely unfit for marketing and processing.

Plot Design: -- See section under corn earworm.

Application Equipment: -- See section under fall armyworm and corn earworm. Basically, when the silk stage is reached application techniques used for earworm apply. The technique recommended for fall armyworm should be used for first generation corn borers, and insecticide sprays, baits, or granules should be applied from an overhead boom directly into the whorl of the plant.

Timing and Frequency of Application: -- A 2-3 day application schedule will be required after silking begins.

Sampling and Evaluation: -- Direct counts of corn borers and borer injury in 25-100 sweet corn plants should be made.

Count the corn borer infestation in 25-100 ears per plot. Observe and record the degree of injury into the same classes used for earworms.

References

Greene, G. L., and J. J. Jones. 1970. Control of budworms on sweet corn in central and south Florida. *J. Econ. Entomol.* 62(2):579-582.

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Hudson, M. 1962. Field experiments with *Bacillus thuringiensis* and chemical insecticides for the control of European corn borer, *Ostrinia nubilalis*, on sweet corn in southwestern Quebec. *J. Econ. Entomol.* 55(1): 115-117.

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Janes, M. J. 1975. Corn earworm and fall armyworm: Comparative larval populations and insecticidal control in sweet corn in Florida. *J. Econ. Entomol.* 68(2): 657-658.

McWhorter, G. M., E. C. Berry, and L. C. Lewis. 1972. Control of European corn borer with two varieties of *Bacillus thuringiensis*. *J. Econ. Entomol.* 65(5): 1414-1417.

Oatman, E. R., I. M. Hall, K. Y. Arakawa, G. R. Platner, L. A. Bascam, and C. C. Beegle. 1970. Control of corn earworm of sweet corn in southern California with a nuclear polyhedrosis virus and *Bacillus thuringiensis*. *J. Econ. Entomol.* 63(2): 415-521.

Irish Potatoes

Irish potatoes, *Solanum tuberosum*, are attacked by two species of Lepidoptera which could possibly be controlled with entomogenous bacteria or baculoviruses. These are the potato tuberworm, *Gnorimoschema operculella* (Zeller) and European corn borer, *Ostrinia nubilalis* (Hubner). Test methods for these insects will be described below.

Potato tuberworm, *Gnorimoschema operculella* (Zeller)

Plot Design: -- For early tests, plots may be 3 beds wide by 2.5 m long. Plots this size will help to prevent normal movement of adults from adversely affecting results under normal population pressures.

For commercial trials, size of the plot is dictated by available ground applications which treat 6-8 beds per swath. Therefore, plots should be 18-24 beds wide by 60 m long. This will also provide enough area in the middle of the plot to take representative samples.

Plots for aerial application should be 36 m wide or 3 swaths of 12 m in width to minimize drift and allow sufficient area to harvest a representative sample of tubers.

Application Equipment: -- A broadcast boom with flat fan tips (8004 is suggested) arranged equidistant should provide optimum coverage when using ground equipment. Knapsack sprayers will give satisfactory results in small plot tests. Full coverage should be stressed with this method and the use of flat fan nozzles is preferred.

Finished spray volume is dependent on plant size but satisfactory results can be expected with ground applications of from 561-935 l/ha. Aerial spray rates of from 93-140 l/ha are recommended.

Timing and Frequency of Application: -- Control of tuberworms requires preventative-type treatments and sprays should be applied on 5-7 day and 10-14 day schedules. This will permit observations on residual activity of the pathogen.

Sampling and Evaluation: -- Normal harvest procedures for the area in which the test is being conducted should be followed.

Harvested tubers should be selected from the center of the experimental plots. Total numbers of tubers selected should be in the range of 500-600 regardless of the total number of plots.

Tubers should be examined and recorded as damaged if a tuberworm mine is found. The number of mines per tuber is not important since one is sufficient to necessitate culling.

The total number of tubers infested is recorded as well as the percent of tubers infested. Care should also be taken to separate "green tubers" (those that are exposed on the surface) from the "marketable" tubers when recording those that are infested.

References

See citations following Irish Potato section.

European corn borer, *Ostrinia nubilalis* (Hubner)

European corn borer attacks both spring and fall crops of Irish potatoes in Virginia and the Atlantic seaboard states. Although pathogens are not particularly effective against this insect because of its feeding behavior, the method described below may be acceptable for test purposes.

Plot Design: -- See section under potato tuberworm.

Application Equipment: -- See section under potato tuberworm.

Timing and Frequency of Application: -- Treatments must be preventative in nature. Criteria which may be used for initiating the spray program are: consistent moth flights as determined by light trap collections, and/or appearance of egg masses in the field. Application should be made on a 5-7 day schedule and must be started before the borers have entered the plant.

Sampling and Evaluation: -- Corn borer infestations are self-evident since the plants either break over or wilt and die. Only one field count will be necessary but at least 5 plants per plot and preferably 10 or more will have to be dissected.

Insect data may be taken as number of plants injured by corn borer or number of borers per plant or plants.

Supporting data should consist of yields or specific gravities. Evaluate as U.S. No. 1 or U.S. No. 2 grade. Yields converted to kg/ha or cwt/acre should be taken from the whole plot or a representative portion therefrom. Secondary effects on tubers such as stem and discoloration, reduced size, etc., should also be recorded.

References

Hofmaster, R. N., R. L. Waterfield, and J. C. Boyd. 1967. Insecticides applied to the soil for control of eight species of insects on Irish potatoes in Virginia. *J. Econ. Entomol.* 60(5): 1311-1318.

Reed, E. M., and B. P. Springett. 1971. Largescale field testing of a granulosis virus for the control of the potato moth *Phthorimaea operculella* (Zell). (Lep., Gelechidae). *Bull. Entomol. Res.* 61: 223-233.

Shorey, H. H., A. S. Deal, R. L. Hale, and M. J. Snyder. 1967. Control of potato toberworms with phosphamidon in southern California. *J. Econ. Entomol.* 60(3): 892-893.

Peppers, *Capsicum annum*

European corn borer, *Ostrinia nubilalis*

Corn borer is the primary lepidopterous pest of peppers. Because of its biology on this vegetable it is extremely difficult to control with chemicals and entomopathogens. Results with microbial agents under optimum conditions have been favorable. Because of the mode of action of microbials there is little point in testing against this pest and no specific method will be reviewed. The reader is referred to the General Methods section and Crucifers section for test design information. The following publications are recommended.

References

Burbutis, P. P., D. J. Fieldhouse, D. F. Crossanan, R. S. VanDenburgh, and L. P. Ditman. 1962. European corn borer, green peach aphid, and cabbage looper control in peppers. *J. Econ. Entomol.* 55(3): 285-288.

Burbutis, P. O., R. S. VanDenburgh, D. F. Bray, and L. P. Ditman. 1960. European corn borer control in peppers. *J. Econ. Entomol.* 53(4): 590-592.

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Snap Bean, *Phaseolus vulgaris*; Lima Bean, *Phaseolus lunatus*; Southern Pea, *Vigna sinensis*; Celery, *Apium graveolens dulce*

Cabbage Looper, *Trichoplusia ni*

The primary lepidopterous pest of this group of vegetables is the cabbage looper, *Trichoplusia ni* (Hubner).

Method description and design to control this pest on these crops can be found in the General Methods section and Crucifers section.

Pome and Stone Fruits and Tree Nuts

The evaluation of baculoviruses and entomogenous bacteria for efficacy and usefulness on fruit and nut crops has been rather limited. The basic approach for conducting required field testing is described in the General Methods section. Additional information is provided below and selected examples of methods employed by researchers for specific pests are included with appropriate referencing.

DECIDUOUS POME AND STONE FRUITS

The most significant pest groups in deciduous tree fruits which are known to be susceptible to control with bacterial and baculoviral insecticides are listed below.

Chewing Insect Pests	Twig Borers
Codling Moth *Laspeyresia pomonella* (Linnaeus)	Peach Twig Borer *Ansaria lineatella* (Zeller)
Oriental Fruit Moth *Grapholitha molesta* (Busck)	Oriental fruit moth *Grapholitha molesta* (Busck)
Fruittree Leafroller *Archips argyrospilus* (Walker)	Wood Borers
Redbanded Leafroller *Argyrotaenia velutinana* (Walker)	
Tufted Apple Budmoth *Platynota idaeusalis* (Walker)	Peach Tree Borer *Sanninoidea exitiosa* (Say)
Variegated leafroller *Platynota flavedana* (Clemens)	Lesser Peach Tree Borer *Sananthedon pictipes* (Grote and Robinson)
Obliquebanded leafroller *Choristoneura rosaceana* (Harris)	American Plum Borer *Euzophera semifuneralis* (Walker)

Chewing Insect Pests

Plot Design: -- Small-scale testing of microbial agents should employ a minimum of 4 single-tree replicates per treatment in a randomized block design. In some situations, buffer trees to prevent drift of spray from one treatment to another may be desirable. Tree size and planting distance should allow each tree to be treated as a unit. Varieties chosen should be typical of those common to the area and susceptible to injury. A standard treatment (one which has a background of information on its performance) and an untreated check plot should be included. The check plot is needed to determine the magnitude of the insect infestations on which the microbial agent is being tested.

Orchards used in large-scale tests must be representative of the area in such matters as varieties, ages of trees, cultural practices, and insect populations. Plots may be replicated in one orchard or in different orchards. The effectiveness of experimental agents can be compared to a standard insecticide product applied in an adjacent area of the orchard. Untreated check plots are required for a valid comparison of treatment results.

Application Equipment: -- A portable, high-pressure sprayer equipped with a pump capable of delivering 10-35 gpm (38-132 l) at 30-60 psi, a single or multi-compartmented tank, high-pressure sprayhose and adjustable individual spray guns are commonly used for small-scale orchard tests. Where test trees are uniform in size, a spray-mast fitted with spray guns may be substituted for the spray-hose and individual spray guns. The tank should be designed for easy rinsing and if divided into compartments, pipes and valves must be arranged to limit delivery and throw-back of spray mixture to and from the pump from only one compartment at a time. Upon changing spray output from one compartment to another, the new spray mixture should be directed to the ground for 15 seconds to clear the pump, hose and gun(s) of previously used spray mixture.

Low volume sprays may be applied using a portable airblast sprayer equipped with a 100-gallon (378.5 liters) tank or larger, a pump capable of operating at 200 psi or higher (lower on highly specialized equipment) at a capacity of 20 gpm (75.7 liters) or more and an air delivery equal to or greater than 20,000 cfm at a velocity of 80 mph (128.7 km) can be used for large-scale orchard tests. (Sprayers with 2.5 times or more air delivery produce more repeatable results. Smaller equipment may be used in some plantings.)

When changing the delivery of spray from one compartment to another in a multi-compartmented sprayer, the sprayer should be operated in an area away from test plots to clear previously used pesticides from pump and lines.

Timing and Frequency of Application: -- Timing of application can be determined by monitoring insect activity. The use of traps (bait pan, light traps and pheromone traps) has been successfully employed to provide information on the activity and abundance of some moth species including codling moth and oriental fruit moth. Techniques for monitoring oviposition and egg hatch have been developed for the codling moth. Computerized programs which forecast the occurrence of life stage events for codling moth are useful for determining when applications can be made for maximum effectiveness (Pickel 1976).

Sampling and Evaluation: -- A primary method for determining the effectiveness of a candidate microbial agent against a chewing insect pest is to determine fruit injury. The status of control may be estimated any time during the season and at harvest by scoring the injury on a sample of fruit from each replicate. Both fruit in the tree and on the ground should be sampled. In some cases estimates of fruit load per tree or other unit area provide useful information in determining impact of the pest and usefulness of the microbial agent. Samples taken too long after an application may permit other factors besides the effectiveness of a pathogen to interfere with the results. As infestations may differ according to variety, separate records should be maintained.

Each fruit should be examined individually for evidence of insect injury. For codling moth or oriental fruit moth each fruit should be cut open and the extent of damage recorded (deep entry - worm, or a shallow entry - sting).

To test microbial agents against fruittree leafroller, employ a mimimum of 6-tree sub-plots replicated 3 times. The sample unit should consist of 100 fruit spurs per sub-plot (post-bloom) or 300 fruit spurs per plot. The number of live larvae found by examining the fruit spurs should be recorded and the results reported as the number of larvae per 300 clusters. At harvest the sample unit should consist of all the fruit from the 2 center trees in each sub-plot. The number of fruit per tree and the number of fruit per tree damaged by the fruittree leafroller should be recorded. Results should be reported as the percent of injured fruit.

Additional information may be obtained in the case of the redbanded leafroller, the obliquebanded leafroller, the tufted apple budmoth, and the variegated leafroller by counting the egg masses on a predetermined number of oviposition sites per treatment. With some of these pests, timed counts of larval feeding sites or examinations of a predetermined number of fruit clusters for larvae may also be of value.

A more complete evaluation of the effect of a microbial agent on insect species infesting deciduous fruit trees can best be made by following the pest situation during an entire season. In this way the effect of a candidate material can be tested for effectiveness on the complex of pests in the test area.

Sampling and counting methods in the large plots are similar to those used for small plots. In those situations where replication is not possible, collect data from at least 10 trees or from several bulk bins throughout the treated area. Compare means and standard deviations to determine efficacy of candidate materials.

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Twig Borers

The larvae of the peach twig borer burrow into tender new shoot growth about the time the first peach leaves appear. The injury results in the death of the terminal and is accompanied by an exudate of gum from the site of injury. Larvae also attack the fruit, usually at the stem end where the feeding excavations become filled with gum mixed with frass.

Oriental fruit moth eggs are laid on the underside of leaves at or near the time peaches are in bloom. After the larvae hatch they burrow into tender new twig growth near the base of the terminal bud. There may be several generations a year, and when succulent twig growth is not available the larvae may attack the fruit.

Plot Design: -- The plot design for twig borers is the same as that used for chewing insects.

Sampling and Evaluation: -- Injury by these pests should be recorded as the number of damaged terminals per tree (peach twig borer dormant treatments or foliar sprays) or as percent injured fruit (foliar sprays only). A minimum of 100 fruits per replicate should be selected at random for each examination. Where evaluation is based on damaged terminals, determine which species caused the damage. The same techniques are used for large-scale field tests, but a larger fruit sample should be taken.

References

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Trunk Borers

The larvae of the peach tree borer and the American plum borer feed at or below ground level and may girdle the trunk. This may result in the death of young peach, nectarine or apricot trees in a single season if several borers are feeding. The first evidence of injury is frass on the trunk of the tree in the early fall. The following spring, an exudate of frass mixed with gum will be evident at the base of the tree.

The lesser peach tree borer restricts its feeding to the larger scaffold limbs of the tree and is inclined to inhabit large pruning wounds or other similar suitable points of entry. Several larvae may develop at a single site and limbs or whole trees may be killed by their feeding. Secretions of gum mixed with frass at the site of injury clearly indicate the presence of these borers.

Plot Design: -- Usually 6-10 trees per treatment in small-scale tests, randomly selected, should be included in each treatment and the check. The possibility of tree mortality due to injury by the peach tree borer may make it impractical to establish large-scale plots for testing candidate pathogens in orchards other than those that have been abandoned for commercial ventures. The same consideration may preclude using large untreated check plots in the experimental design. Depending on the microbial agent used, it may be advisable to make repetitive tests for at least 2 seasons in order to get meaningful results with peach tree borers, particularly with small-scale tests where total numbers of insects will be very small.

Application Equipment: -- An adjustable handgun attached to a high-pressure hydraulic sprayer that can deliver up to 35 gpm (132.5 l) at from 200-600 psi can be used for application. The candidate microbial agent should be applied uniformly over the target area until it has been thoroughly wetted. For the peach tree borer, the spray material should be directed to the trunk of the tree. In the case of the lesser peach tree borer, it is important that the trunk and the larger limbs be thoroughly sprayed. Accurate measurement of quantity of pathogen applied per tree or per hectare related to the diameter of the tree trunk at a predetermined height is reported.

Timing of Application: -- Timing of applications should be correlated with the seasonal development of the target pest, so that the susceptible life stage is present when treatments are applied. Male moth emergence can be monitored with pheromone traps and other developmental information may be obtained form close observation of caged or field populations.

Sampling and Evaluation: -- The appropriate pheromone may be used to determine commencement, duration, intensity and termination of male moth activity. Moth catches should be recorded in such a manner that the number of trapping days included in each recording is clearly indicated. Weather information is included whenever possible.

Evaluation of candidate microbial agents for control of boring insects requires that a detailed examination of the trunk and larger limbs be made in the late fall following the application of spray treatments. Data for the treatments and control should be recorded as live larvae per tree.

Preliminary population level estimates of peach tree borer and lesser peach tree borer on each tree may be obtained by counting fresh frass poles in the fall (peach tree borer, American plum borer) or in the summer (lesser peach tree borer), excavating larvae from infested trees, and also counting (weekly) the number of cast pupal cases extending from the bark once the moths begin to emerge. Each pupal case should be destroyed once it has been recorded to avoid overestimating the population. Data should be presented as cast pupal cases per tree.

If soil applications of pesticides as surface sprays are made, the components of the vegetative cover should be noted and the pH of the soil determined and recorded.

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TREE NUTS

The following list presents the most significant pest groups which are known to be susceptible to bacterial and baculoviral agents. Test methods and supporting information for the evaluation of pathogens are described only when they differ from the General Methods section.

Chewing Insect Pests	Twig Borers
Codling Moth (Walnut) *Laspeyresia pomonella*	Peach Twig Borer (Almond) *Anarsia lineatella*
Filbertworm (Walnut) *Melissopus latiferreanus*	Tree Borers
Navel Orangeworm (Almond) *Paramyelois transitella*	Peachtree Borer (Walnut) *Sanninoidea exitiosa*
Hickory shuckworm (Pecan) *Laspeyresia caryana*	Defoliators
	Redhumped caterpillar (Walnut) *Schizura concinna*

Chewing Insect Pests — Codling Moth and Filbertworm — Walnut

Plot Design: -- A minimum of 5 single tree replicates in random distribution is recommended.

Sampling and Evaluation: -- The sample unit should consist of 100 nuts per replicate randomly collected from the ground at strategic times during the season and from the tree at harvest. The nuts are cracked to determine the number damaged by codling moth larvae and the results reported as the percent of nuts infested by codling moth.

References

Falcon, L. A. 1970. Field studies with *Bacillus thuringiensis* for the control of codling moth on walnuts in northern California. *Progress Report*. University of California, Berkeley. 18 pp.

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Navel Orangeworm —— Almond

Plot Design: -- A minimum plot size of 1 acre should be used.

Sampling and Evaluation: -- 10 trees should be selected from the center of the plot. The sample unit for the treatment should consist of 100 nuts taken from each of the 10 trees. The nuts in the composite sample should be hulled and shelled and a determination be made as to whether the navel orangeworm has attacked the nut meat. Results should be expressed as the percent of kernels damaged by the larvae. If an untreated plot is included in the test, the results should further be expressed in terms of the percent reduction of damaged kernels.

References

Pinnock, D. E., and J. E. Milstead. 1972. Evaluation of *Bacillus thuringiensis* for suppression of navel orangeworm infestation of almonds. *J. Econ. Entomol.* 65: 1747-1749.

Summers, F. M., and D. W. Price. 1964. Control of navel orangeworm. *Calif. Agric.* 18(2): 14-16.

Hickory Shuckworm —— Pecan

Plot Design: -- A minimum of 8 single tree replicates in random distribution should be used.

Sampling and Evaluation: [illegible] The sample unit should consist of 50 shucks per tree sampled. A determination should be made as to whether the shuck is infested and the results expressed as the percent of shucks infested. Ancillary data should be obtained with regard to the number of nuts per pound, based on a random sampling from the harvest of each count tree. The results should be expressed as the average number of nuts per pound for the treatment.

References

Osburn, N. R. 1954. EPN for control of the hickory shuckworm on pecan. *J. Econ. Entomol.* 47(5): 931.

Payne, J. A., W. L. Tedders, and C. R. Gentry. 1971. Biology and control of a pecan serpentine leafminer, *Nepticula juglandifoliella*. *J. Econ. Entomol.* 64(1): 92-93.

Twig Borers —— Peach Twig Borer —— Almond

Plot Design: -- A minimum of 10 single tree replicates in random distribution should be used. The trees should be 2-5 years of age.

Sampling and Evaluation: -- The number of worm-damaged terminals ("strikes") found on each tree counted is recorded. If the treatments are applied before bloom or during the petal-fall period, the counts should not be made until the surviving overwintering generation larvae have matured. If the treatments are directed against the next generation, counts should be delayed until early summer. The results should be reported as the number of "strikes" per plot of 10 trees.

On the older bearing trees the extent of damage to nuts should be determined. At harvest, a 30-pound (13.5 kg) sample of nuts should be randomly selected from each treatment plot. The nuts should be hulled and cracked and the number of wormy meats and the total number of nuts examined recorded. Results should be reported as the percent of injury to nuts.

References

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Summers, F. M. 1951. Tests of new materials to control peach twig borer on almonds and peaches. *J. Econ. Entomol.* 44(6): 935-939.

Tree Borers —— Peachtree Borer —— Walnut

Plot Design: -- A minimum of 5 trees per sub-plot, replicated 5 times in random distribution, should be used.

Sampling and Evaluation: -- Each tree of the treatment group should be examined during the spring period following applications made the previous year to determine the number of live borers present. Results should be expressed as the number of live borers per tree. See also Peachtree Borer in Deciduous Pome and Stone Fruit section.

References

Snapp, O. I. 1962. Peach tree borer experiments in peach orchards. *J. Econ. Entomol.* 55(3): 418-419.

Defoliators —— Redhumped Caterpillar —— Walnut

Plot Design: -- Single tree plots with up to 6 trees may be used. However, plot design may vary according to the host tree and larval density.

Application Equipment: -- All formulations are applied to the point of runoff; the volume applied per tree depends on the surface area of foliage treated and may exceed 60 l per tree.

Timing of Application: -- The pathogen to be tested is applied when the majority of the larval population is in the 1st to 3rd instar. In these stages the larvae are gregarious and easily detectable, and the defoliation sustained by the host tree is kept to a minimum.

Sampling and Evaluation: -- Trees are individually coded so that populations of larvae may be monitored on a tree-by-tree basis. At each observation time, total larval counts classified by instar are recorded for each tree by pooling results for all broods found on that tree.

References

Pinnock, D. E., J. E. Milstead, N. F. Coe, and R. J. Brand. 1974. The effectiveness of *Bacillus thuringiensis* formulations for the control of larvae of *Schizura concinna* on *Cercis occidentalis* trees in California. *Entomophaga* 19: 221-227.

GREENHOUSE PLANTS

Crops grown in the greenhouse are either ornamentals grown in pots or beds, or vegetables grown hydroponically or in soil. In either case, the crops and the greenhouse have several unique characteristics which can greatly influence the determination of levels of efficacy and testing procedures. Perhaps the most important of these is the extreme susceptibility of greenhouse plants to phytotoxic reactions. This factor is important enough that separate tests may be necessary in which all stages of plant growth are exposed to each formulation of microbial insecticides. Another important characteristic of greenhouse crops which has many ramifications is that they generally have a much higher value than field crops, which causes a lowering of economic injury levels. In the case of ornamentals, the market dictates that this level is zero, that is, both a flower and its attached foliage must be free from damage.

High value of crop and space under glass also affects application and testing. In maximizing the utilization of space, plants are crowded together and aisles between beds are kept so narrow that the choice of spray equipment is limited. Testing is affected by the low tolerance for damage. A pathogen must be able to prevent damage, but in order to obtain significant efficacy data, a high pest population must be allowed to develop.

A third characteristic of the greenhouse is that crops are usually in all stages of growth. This requires that workers handle the crops continuously and thus their exposure time to the candidate material will be very high. This may be an advantage for microbial agents.

There are other factors which can affect application and testing. High humidity may cause a fungus problem which can require frequent application of fungicides. Fungicides may interfere with the microbial agents applied for insect control. Frequent overhead watering can wash off the microbial agent. Temperatures are moderate and probably optimum for the growth of most insect pests and microbial agents. Ultraviolet light radiation may be decreased, which is beneficial to the survival of the microbial agents.

Microbial pest control efforts in the greenhouse have been extremely limited; Lepidoptera have been the primary targets for control using entomogenous bacteria.

General Methods

Application Techniques and Equipment

Pathogens may be applied with any of the following techniques:

- High or low-volume pressure sprayer and handgun. The spray should be put on to the point of runoff. High volume sprays may be simulated by dipping plants into spray mixtures.

- Aerosols. The material cannot be viscous or particulate.

- Ultra low volume —— microdroplets. Cold fogging applicators must be used. If highly viscous or impure preparations are used, an air-shear, venturi nozzle would be most appropriate to avoid clogging. Tests should be conducted to determine coverage in dense foliage (see General Methods section).

- Dusts.

- Contaminated mobile pests.

Efficacy Testing

Plot Design: -- Individual plot size will vary depending on the type of application, type of pest and pest population. Plots can consist of single potted plants, an entire bed or section of the greenhouse, or an entire greenhouse. In general, the smaller the plot size, the larger the number of replications that will be needed. Single plants should be replicated at least 10 times. When using foggers or aerosols, each greenhouse will constitute one plot. However, it is sometimes possible to cover beds or plots with plastic to provide an untreated check. In all tests, the smallest practical plot size should be used. To avoid cross-contamination, polyethylene plastic tarps should be placed between plots. Replicated untreated controls must be included and contaminated insects should be prevented from wandering into adjacent plots.

Sampling: -- Damage to the crop must be assessed, as well as pest mortality. Pretreatment counts should be made to establish level of infestation. Lepidopterous pests can be sampled by removing foliage or flowers and examining them under a dissecting microscope. This method has the drawback of removing pests from the population before they succumb to the pathogen. An alternative method is the time-count method. In this method, the searcher examines as much foliage as can be sampled in a given amount of time. 10 minutes for 10 feet of plants is the minimum amount.

Sampling Interval: -- Because pathogens are slow-acting, counts for mortality should be done no sooner than 7-14 days after treatment. Pathogens, however, may act as repellents or feeding suppressors, and to detect these types of activity, a count should be made within 24 hours.

Small-Scale Tests: -- Small-scale tests should be used wherever possible to determine the efficacy of the pathogens and any possible phytotoxic reactions.

Large-Scale Tests: -- Large-scale plots are for demonstrating the ability of pathogens to prevent economic damage.

Specific Insects and Crops

Armyworms of several species including the fall armyworm, *Pseudaletia unipunctata* (Haw.) and the yellow striped armyworm, *Prodenia ornithogalli* Guen, often infest greenhouse vegetables in the fall season. Night flying moths of the corn earworm or tomato fruit worm enter the greenhouses in late summer or early fall and lay their eggs on tomato foliage. The young larvae feed at first on the foliage; later, they cut small entrance holes in the fruits and devour the interior. A single larva may damage several fruits. The cabbage looper, *Trichoplusia ni* (Hbn.) moths enter greenhouses in the fall. This insect is probably more widespread and the most generally serious caterpillar pest of greenhouse vegetables and ornamentals. If uncontrolled, cabbage loopers continue to breed on greenhouse crops throughout the winter. Cabbage loopers feed on foliage of tomato, cucumber, cress and radishes and are especially damaging to lettuce where they are also most difficult to control. Prompt and regular applications of an effective insecticide are essential.

The early larval instars of armyworms, cabbage loopers, corn earworms and other lepidopterous pests are more susceptible to microbial agents than are older larvae. Early detection of infestations and prompt treatment in greenhouse vegetable crops are important for efficient control.

As the pest species discussed above are also important pests of outdoor commercial vegetable crops in many parts of the country, data on promising new microbial agents that results from field experiments should give leads to materials that may be adaptable to greenhouse crops.

Plot Design: -- Grow lettuce, tomato, cucumber or other plants in 10-15 cm (4-6") pots in isolation to prevent infestation by unwanted pests. To obtain caterpillars of each species, infest groups of plants with eggs from moths reared from local infestations or captured in black lights, or hold eggs for hatching and transfer known numbers of larvae to plants one day before applying the test insecticide. Use larvae of same age, stage of development and weight.

For the initial tests, groups of 4 or more pots of plants containing a total of 50 or more larvae in each age group are treated as a unit for each dosage level and within the range of greenhouse temperatures required for production of the crop involved. 3 or more replications per treatment are required for analysis of results. Include untreated controls, and if possible, treatment with a product of known performance.

Application and Equipment: -- Sprays should be utilized for control of localized infestations of these pests. Knapsack sprayers operating at 2.1-4.2 kg per cm^2 (30-60 psi) and delivering 187.1-935.4 l/ha (20-100 gal/

acre) are satisfactory for preliminary tests on infested plants. For application of aerosols, individual compartments with volumes of 28.3 m^3 (1000 ft^3) or more may be required. For these tests, which would precede treatments in larger greenhouses, groups of plants infested as for preliminary spray tests could be placed throughout the compartment to provide infestations for mortality counts. All tests should be conducted within the temperature range required for proper growth of the plant and at a time of day or night when host plant injury might be critical due to closing of ventilators. As sick caterpillars usually drop from treated plants, sheets of polyethylene or other material may be placed on the soil or mulched surface around the plants before applying the test chemical.

Make direct counts on a representative sample of plants from each replicate. Make insect injury ratings of 1-5 or 1-10 on host plants and carefully describe leaf damage such as percentage of leaf area destroyed or market acceptability for each category. Record number of injured and uninjured fruits in treated and check plots. Record yield of treated and untreated plots and make note of the extent of feeding injury to marketable parts of the plants. Record host plant injury following application of test material, including foliage injury as chlorosis, marginal burn, and also flower bud abscission on tomatoes and cucumbers.

References

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Tomato Pinworm

The tomato pinworm, *Keiferia lycopersicella* (Busck), has a long history of sporadically infesting greenhouse tomatoes in northern states and is a regular pest on greenhouse tomatoes as well as field-grown tomatoes in warmer parts of the United States. The tomato pinworm does not survive out of doors in northern states but infests field-grown tomatoes near greenhouses where infestations persist from year to year. It is transported to new areas on infested tomato plants or in fruits or in used containers.

Larvae make blotch mines in leaves, feed in growing tips and flower buds, and enter the fruit through pinholes under the calyx of ripening fruit. Effective control efforts should be directed toward the insect before it invades the fruit where it is difficult to control with a microbial agent but causes the greatest damage to the tomato crop.

Crop and Location: -- Select a variety of tomato commonly grown in commercial greenhouses. Plants should be grown in containers and under isolation to prevent unwanted infestation with pinworms and other insects. Less desirable for preliminary tests is the selection of plots in infested greenhouses where active flying adults can reinfest treated as well as untreated plants.

Plot Design: -- Groups of plants infested with 50-100 or more insects for each treatment should be replicated 3 or more times. Each series of tests should include comparable groups of plants that receive (a) a known effective treatment as standard, and (b) no treatment. Treated and untreated groups of plants should be isolated to prevent posttreatment reinfestation.

Application and Equipment: -- Expose plants for 1-2 days to egg-laying adults in a cage or greenhouse compartment that contains infested plants. Application of the test materials should be made to groups of plants prior to egg hatching to determine their potential for destroying larvae before they penetrate the leaves or fruit.

To test the effect of materials against larvae within leaf mines, make application to plants when the larvae are in their early instars and the mines are small, and against more mature larvae when mines are larger.

After the effectiveness of the candidate microbial agent has been determined as above, a series of treatments at timed intervals can be made to plots or preferably to entire sections of commercial greenhouses.

Sampling: -- For determining effectiveness against hatching larvae, count and examine the leaf mines, as they will indicate larvae that survived the treatment. Make direct counts of older larvae in mines or adults in cages at 7 and 14 days after treatment.

In tests conducted on a growing crop in a commercial situation where 2-8 or more applications per week were made, record the number of dead larvae in mines, the number of mines per leaf on treated plants, and the number of fruits with pinworm injuries.

References

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Cutworm

Several species of cutworms including the black cutworm, *Agrotis ipsilon* (Hufnagel); varietaged cutworm, *Peridroma saucia* (Hubner); and dingy cutworm, *Feltia subgothica* (Haw.) attack lettuce, cucumbers, tomatoes and cress, especially in the seedling stage. The variegated cutworm and others known as climbing cutworms also climb older plants and feed on leaves, buds and fruit. All hide in the soil or mulch during the day and feed at night. In the greenhouse, adults and young larvae have been controlled by aerosols containing parathion or malathion. Baits containing bran, molasses, sometimes other ingredients, and a toxicant are effective against older cutworm larvae.

Plot Design: -- For tests in greenhouses with cutworm infestations on lettuce, tomato, cucumber or cress, select plots of adequate size to reduce influence of larval dispersal from contiguous plots. If facilities are available, cutworm larvae may be reared and released in plots 1 or 2 days before treatment.

Application and Equipment: -- Bran baits prepared with or without molasses and with the test compound as toxicant may be used. These are broadcast late in the afternoon at a rate of 11.2-22.4 kg per ha (10-20 lb/acre). It should not be scattered on the plants, but directed to the ground.

Sampling: -- Make direct counts of dead larvae found in soil depressions around the base of injured plants. Make counts of cutworm-injured plants at 1 and 7 days posttreatment.

References

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FORAGE CROPS

Forage crops and principally alfalfa and clovers are attacked by a wide variety of insects, but only a few are known to be susceptible to infection by entomogenous bacteria or baculoviruses. These are all foliage feeders and are similar in enough respects that general methodologies for determination of microbial agent efficacy may be applied to all with specific details pertinent to each being covered under a discussion of the individual pests. In addition to the specific test methods listed below, the reader is referred to the introductory section of this document for general considerations applicable to the efficacy testing of all baculoviruses and entomogenous bacteria.

Crop Variety and Location of Tests: -- The variety tested should be one recommended for cultivation in the test region. It should be susceptible to damage by the insects to be controlled. If mixtures of legumes are planted, a common agronomic practice, the percent stand composition of each species should be determined. Plots should be located in areas which have a history of past infestations of the desired pest.

Experimental Design: -- A randomized complete block design is appropriate for determination of efficacy of microbials on lepidopterous defoliators of alfalfa, clovers, and other forage crops. Plot size for most work with viruses and *Bacillus thuringiensis* has been relatively large, i.e., 6-9 m wide by 45-120 m long for ground application plots and three swath widths by 300-450 m long for aerially applied formulations.

Most caterpillars do not move laterally to significant extents unless populations are so high that complete defoliation occurs. Thus the plot sizes above should be adequate from this standpoint if sampling is confined to their central portions.

Application and Equipment: -- Entomogenous bacterial formulations and baculoviruses can be applied in initial, small plot tests by compressed air sprayers or low pressure ground apparatus. Aerial applications can be applied with standard fixed wing aircraft or by helicopter. Volumes of final formulations applied have been in the range of 15-95 l/ha for nuclear polyhedrosis virus and *Bacillus thuringiensis* applied by ground equipment and 48-95 l/ha when applied aerially.

Sampling: -- Populations of Lepidoptera on alfalfa and clover are normally sampled with the standard 15-inch sweep net. One sweep is considered to be a 180° arc made horizontally over the crop. The number of sweeps should be adjusted to the population levels so numbers of insects obtained are valid for statistical analysis.

Samples should be taken immediately prior to spray applications and again at 1, 3 and 7-day intervals post-application. The residual effects of viruses may require that additional samples be made on the same plots for several host generations. Such samples should be compared to adjacent untreated plots and larvae collected should be examined to determine the presence of virus in the tissues.

For certain insect-microbial interactions, mortality may not provide an accurate assessment of efficacy. In such cases, yield of hay per unit area may be a more valid measure of efficacy.

Analysis and Reporting of Data: -- Both insect counts and yield data should be subjected to analysis of variance tests and treatment means compared by multiple range tests. In addition, the results of laboratory tests on percent incidence of the pathogenic agents in field-collected larvae, changes in insect counts over time, plant height and amount of defoliation at time of treatment, and standard weather parameters should be reported.

Alfalfa Caterpillar

The alfalfa caterpillar, *Colias eurytheme* Boisduval, is principally a pest of alfalfa in southwestern United States and the alfalfa growing regions along the Pacific slope. It only occasionally damages clover and other legumes. In the southwest, it may have from 5-7 generations per year. It is highly susceptible to *Bacillus thuringiensis* infection and is also susceptible to a nuclear polyhedrosis virus. Methods for determining efficacy of these agents conform to those described above.

References

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Green Cloverworm

The green cloverworm, *Plathypena scabra* (Fabricius) is an occasional pest of alfalfa and clover although it is almost always present on these crops. It frequently causes economic damage to other crops such as soybeans and other legumes and is most serious in the southeastern states. It is highly susceptible to *Bacillus thuringiensis* and is also infected by a granulosis virus. Methods for determining efficacy for these agents of forage crops conform to those described for forage defoliators above.

Alfalfa Webworm and Garden Webworm

The alfalfa webworm, *Loxostege commixtalis* (Walker), and the garden webworm, *L. similalis* (Guenee), can severely damage alfalfa, and they occasionally reach pest status on clover. Both form webs over leaves and consume these leaves under cover of the webs. Found throughout the United States, they may have from 2-5 generations per year depending on the latitude. Both insects are susceptible to *Bacillus thuringiensis* and the methods for determining efficacy as described for lepidopterous forage pests in general are also applicable here.

References

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Introduced European Skipper

A pest of southern Canada as well as northcentral and northeastern United States, the introduced European skipper, *Thymelicus lineola* (Lepidoptera:Hesperiidae) damages many hay and pasture crops, principally grasses. Larvae feed on leaves of these crops, severely reducing yields. The methods utilized for determination of efficacy are essentially the same as described for forage crops in general. It has been shown to be highly susceptible to *Bacillus thuringiensis* under field conditions.

References

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RANGELAND

A discussion of the problems associated with control of pests on rangeland has been prepared and published in a previous report (AIBS. 1977. *Analysis of Specialized Pesticide Problems, Invertebrate Control Agents Efficacy Test Methods: Volume II —— Foliar Treatments II.* Report to the Environmental Protection Agency EPA 540/10-77-009.) An excellent description of the rangeland caterpillar, a pest of rangeland grasses which is susceptible to both bacterial and baculovirus infections, is also presented in that document. Portions of the discussion presented there are repeated here where appropriate. Additional discussion is included only where necessary to modify that text for the demonstration of efficacy of *Bacillus thuringiensis* or baculoviruses. The reader is referred to the introductory section of this document for general considerations applicable to the efficacy testing of all baculoviruses and entomogenous bacteria.

Rangeland pest control presents unique problems because of the vast acreages usually involved and the relatively unfavorable cost-benefit ratios involved in making insect control applications to these crops. Nevertheless, defoliation can at times be so severe that control measures are a necessity if economically feasible methods are available.

The above-mentioned factors, in addition to the rough terrain, usually necessitate aerial application of insecticides in almost all instances. Many of the species of insects which damage rangeland forage are quite mobile and this factor, coupled with the variability in population densities and the necessity of evaluating aerial application, has led to the use of large (20-259 ha) plots for efficacy testing. Plot size often must be reduced in mountain or forest rangeland.

Careful attention should be given to the overall efficacy evaluation program to ensure that the tests in different areas are a part of the same experimental design so that the number of replicates at a given site can be reduced. Sub-plots are often selected at random within the large plots to improve efficiency. Care should be given to selecting plot locations which will not involve spray applications over ponds or water courses unless it is the intention of the researcher to monitor for pesticides in those areas.

Minimum plot size for aerial application will usually be three swaths wide by long enough to assume sustained level flight of the aircraft. Plots must be long enough to allow for variation in initiation of spray at each end. Plot size may be reduced by the use of smaller, slower aircraft. Width of plots or separation between plots must be adequate to prevent drift onto adjacent plots. Preliminary "screening" can be accomplished by low pressure - low volume sprayers. Minimum plot size is dictated by the mobility of the insect species involved. Border treatment with pesticides labeled for the particular use can be used to further reduce plot size.

Microbial agents should be applied with carefully calibrated equipment under acceptable weather conditions. To make certain of this, more than one day may be required for aerial application of several treatments.

Grasshoppers

Grasshoppers are not known to be susceptible to baculoviruses and show little response to *Bacillus thuringiensis*. Therefore, no detailed descriptions of techniques for testing these agents against grasshoppers will be presented here. Information on field testing of chemical insecticides is contained in the previously mentioned Report to the Environmental Protection Agency 540/10-77-009. Many considerations presented in that source would be applicable to field testing of baculoviruses and entomogenous bacteria if and when candidate pathogens are discovered.

Range Caterpillar

The range caterpillar, *Hemileuca oliviae* Cockerell, feeds primarily on range grasses in areas in northeastern and south central New Mexico at elevations between 1734-2438 meters (4,700-8000 feet). The infestation has extended into southeastern Colorado and the western edge of the Texas Panhandle. Range caterpillars consume large amounts of foliage, waste additional unconsumed parts of leaves and cause other foliage to be ungrazed because of the presence of irritating spines on the active larvae and cast skins (Hewitt et al. 1974). Heavy populations may destroy all grass down to the crown, producing conditions conducive to wind and water erosion.

Crop and Location of Tests: -- Species composition of the vegetation and the terrain are usually dictated by the location of economic infestations of the pest. Care should be given to uniformity of vegetation and terrain among treatments. Proximity to watering sites will affect forage utilization and distribution of livestock.

Experimental Design: -- Since migration is limited in the early instars, plot size can be considerably smaller than is required when the caterpillars are large. Migration by large caterpillars is increased as the density of the population and percentage of standing crop foliage consumed increases.

For small worms, plots should be at least three swaths wide by 402.2 m (¼ mile) in length. For larger worms, the plot width should be at least doubled. To reduce drift, plots should be separated by an adequate distance which will depend on wind velocity and direction.

Plots as small as 2.43 hectares (6 acres) have been used for efficacy tests by airplane against first and second instars.

Watts, et al. (unpublished data) has experimented with circular arenas encompassing approximately 4.18 m^2 (5 $yard^2$) to confine known numbers of small caterpillars within the test plots on an experimental basis to reduce variation in density. These arenas were made of 6-inch strips of tin forced ca 2.54 cm (1 inch) into the soil. Larger plots, from 20.25-259.2 ha (50-640 acres) have been used on other studies in New Mexico. Coppeck (unpublished data) conducted preliminary screening test with a compressed air hand sprayer on small plots.

Application and Equipment: -- Aerial application equipment that is designed for the aircraft being used should be properly calibrated. Aircraft designation; boom size and length; number, size of nozzle, or atomizers and position on aircraft; pressure; aircraft speed; altitude and swath width; type of solvent; concentration of solution and quantity of solution used should be reported. Dye cards for evaluation of deposit uniformity are recommended. Flight runs made crosswind usually increase the uniformity of deposits. Applications should be made under conditions that avoid excessive convection currents.

Sampling: -- Population densities are usually evaluated by counting the number of caterpillars in square yard sampling areas located well within the plot. Various sampling schemes, designed to remove bias and assure coverage of an adequate area, have been used. The sampling scheme used should assure that an entire swath width and preferably more be included in the area to be sampled. A minimum of 10.84 m^2 (1 $yard^2$) samples or more, until at least 50 worms are counted, is needed per plot. Additional samples will increase precision. When 5, 4.18 m^2 (5 $yard^2$) arenas are used, 100 caterpillars per arena should be used.

Because of the habits of the range caterpillar, the accuracy of visual counts is increased by delaying initiation of counting until mid-morning when ground temperatures have increased enough to initiate activity in the caterpillars.

Counts should be taken immediately prior to treatment and at intervals estimated to embrace partial and maximum kill.

Analysis and Reporting of Data: -- Where possible, treatment means should be compared using a valid statistical test for significance. The standard material used in private-state-federal control programs in the geographic area should be included. Replicated untreated plots are also recommended.

References

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LAWNS, TURFGRASSES, PASTURES

Lawns and turfgrasses are generally considered high value crops or commodities, principally because of their aesthetic values. Determination of efficacy of microbial agents for control of insect pests of these commodities may, therefore, be conducted on a labor and cost intensive basis because while control costs are usually high on a per acre basis, they are borne by the owners because of the aesthetic values and because total land areas treated are normally small. These commodities may often sustain considerable levels of many pest species before noticeable damage or damage requiring treatment occurs.

Pastures, on the other hand, have a much lower monetary value as a commodity and correspondingly lower amounts of money may be spent for control of pests on them. Economic threshold levels on pasture grasses, however, may be higher than on lawns because yield losses may occur even though unsightly or aesthetically unpleasing damage is not occurring.

While the above differences may dictate different management decisions for pest control, methodologies for determining efficacy of microbials on small plots will be essentially the same. Insect control on lawns, turf and pastures will normally be accomplished with ground equipment. In addition to specific test methods listed below, the reader is referred to the introductory section of this document for general considerations applicable to the efficacy testing of baculoviruses and entomogenous bacteria.

Small-Scale Field Tests

Site Selection: -- The site or sites selected for tests should be of a uniform host plant or of a uniform mixture of host plants. If damage has already occurred, it should be of uniform severity and the pest population should be of uniform density over the entire field plot layout. If such conditions cannot be met, treatments should be limited so that any comparisons are valid and meaningful.

Normally, established lawns, golf fairways and greens, pastures and any other turf will be infested and will provide conditions wherein typical grass varieties for a given locality will be planted. Pest populations should be increasing at the time the tests are conducted. If this is not the case, a measure of population quality should be given. Pretreatment population counts taken just prior to treatment should be made.

If standard management practices are to be employed on the treatment area (e.g., application of fertilizers, growth regulators, fungicides, etc.) they should be applied uniformly over the entire test site. In the case of pastures, some provision may be needed to exclude livestock from grazing on treated plots following the tests if test materials do not have an exemption from tolerance or if season-long or multiseasonal effects are to be monitored.

Plot Size and Design: -- Most turf grass, lawn and pasture pests which are susceptible to *Bacillus* sp. and baculoviruses are not highly motile in their damaging stages. Thus relatively small plots (e.g., 1.5 x 3 to 3 x 3 m) will normally provide satisfactory data. Unusually heavy populations of certain pests may necessitate larger plots to overcome immigration effects whereby the edges of the treated plots would serve as buffer zones with data being taken from the central portions of the plot. Normally a minimum of 4 replicates should be included for each treatment. This number might need to be increased for pests which show discontinuous or contagious distribution patterns.

Application and Equipment: -- Formulations should be applied when the pest is present in sufficient numbers to provide meaningful population reduction and damage estimates when the treatments are evaluated at a later date or dates. Applications should be made with equipment that distributes the formulations in the same manner as would be accomplished using standard application equipment for that area. Every care should be taken to accurately calibrate the equipment and distribution procedure. The latter may simply involve uniform application of pre-measured amounts of material to each plot as an alternative to calibration. Materials should be applied in their commercial formulations where possible.

Dosage Selection, Treated Check, Untreated Check: -- Dosage is normally dictated by the manufacturer, but where possible, it is desirable to include treatments of ½x and 2x the recommended rates. Where no previous data is available, these intervals should be increased or a wider range of treatments should be tested, especially in the first year of tests against a given host.

If the goal of treatment with the microbial insecticide is rapid control, efficacy should be compared to a standard insecticide recommended for use in the test area. Similarly, untreated check plots are an absolute necessity for evaluating the influence of natural infections on the pest population during the relatively long period of time required for evaluation of efficacy of these products. If season-long, multiseasonal or permanent establishment (colonization) of the pathogen is sought, standard insecticidal check plots and untreated check plots should also be included as a basis for comparison of costs of treatments as well as providing an estimate of the pest potential in the absence of treatment.

Number of Trials: -- The number of trials cannot be rigidly established. Sufficient numbers should be included to permit accumulation of data on:

1. Efficacy over the range of the pest for which registration is sought.
2. Persistence or self-perpetuation in the environment.
3. Compatibility with all application systems with which it would be applied.
4. Effects of weather factors on efficacy.
5. Proper timing with respect to:
    - stage of insect host
    - stage of host plant
    - need (under the concept of pest management programs).
6. Effects on non-target insects.
7. Activity in the target insect.

Statistical Analysis: -- The experimental design utilized should be such that a measure in variation of response by the host insect is obtained. At the very least this should be a measure of the standard deviation in the host-pathogen treatment which can be compared to the effects in the untreated host population. When several rates, formulations and products are tested, an analysis of variance with a multiple range test applied to treatment means should be performed.

Sampling Methods: -- Sampling methods vary from crop to crop and insect to insect and will be described for each host plant-pest insect interaction discussed.

Large-Scale Field Tests

For the commodities listed in this section, large-scale tests may be of little value. Most ownerships will involve relatively small acreages with area-limited infestation. Therefore, ground applications using techniques applied to small plots should be applicable to most pest problems.

Webworms

Sod webworms (Lepidoptera:Pyralidae) are pests of lawns and grass in most areas of the United States. Several genera and species are included under the general term sod webworm. The most common pest species are members of the genera *Herpetogramma* and *Crambus*. Sod webworms nip and clip grass blades and pull them into silken tunnels which they construct near the ground line. Under certain weather conditions this damage is noticed as small, circular brown areas over a lawn or in turf. If the infestation is severe enough, such areas may be so numerous as to run together causing large patches of dead grass (Scheibner 1972). Many natural control agents, climatic factors, and cultural methods reduce severity of sod webworm damage. Insecticidal control programs should be implemented to complement action by these other factors in cases where their activity is insufficient to reduce damage below acceptable levels.

Crop Variety and Location of Tests: -- The crop variety will vary considerably in tests on sod webworm since different grasses are utilized as lawns throughout the United States and many are attacked. Thus the variety of crop will normally be that commonly grown in a particular region. In any event, the species of grass or grasses being treated should be clearly identified. Plots are normally located in areas where infestations occur naturally because of the difficulty of establishing and maintaining turf for this specific purpose. Infestation areas should be large enough to allow sufficient replications of all treatments to be established over uniformly infested turf. Populations have been encouraged in some tests by application of fertilizer to maintain a vigorous stand of grass (Reinert 1976).

Experimental Design: -- Sod webworm control tests normally should be established in randomized complete block designs with three or four blocks. Within block variation was reduced in one test (Reinert 1973) by establishing each block in areas of uniform infestation where between block infestation differences were present. This would be acceptable if no uniform test areas are available.

Relatively small plots of 4-5 m^2 may be utilized effectively for determination of efficacy of *Bacillus thuringiensis* because of the low mobility of these insects under normal infestation levels and because treatment of such plots can closely simulate techniques utilized on a commercial or homeowner scale. These plots can be relatively close together with only a small (0.5 m) buffer strip between plots to reduce possible overlapping of spray applications.

Application and Equipment: -- *Bacillus thuringiensis* can be applied uniformly to small plots with a compressed air sprayer. Large volumes of water 1077 l/ha (436 gal/acre) have been used (Reinert 1976) on such small plots. The use of sticking agents may be desirable. Granular formulations can be dispersed by hand shakers and watered into the turf.

Sampling: -- Sod webworm sampling is effected by use of pyrethrins applied to uniform areas within the plots. Normally 0.02-0.13% solutions are applied with a sprinkling can at a rate of approximately 0.95 l/0.36 m^2. Larvae are counted as they come to the surface following treatment and counts are made over a standard time period, usually 10 minutes. Counts should be continued at weekly intervals, and care should be taken to sample different areas within each plot at each sampling date.

Larval counts, if used as the only measure of effectiveness of *Bacillus thuringiensis*, may give misleading estimates of product efficacy. Many sod webworms may ingest *B. thuringiensis* and not be killed immediately if at all, yet feeding activity may be stopped or severely reduced. It is thus important to monitor stand condition, monitoring the rate of continued browning or of "greening up" in both *B. thuringiensis*-treated plots, untreated control plots and plots treated with standard chemical insecticides. Numerical ratings which can be analyzed statistically should be taken for all tests of *B. thuringiensis* efficacy. Because of this mode of action, evaluation of *B. thuringiensis* should be continued at least as long as the generation treated is still present in the field and new damage from progeny of immigrating moths does not interfere with assessment of residual effects on the population originally present.

Analysis and Reporting of Data: -- In addition to data listed in Reporting Microbial Agent Test Report, the following should be reported:

- Larval counts per unit made immediately prior to spraying and at weekly intervals following treatment.

- Stand condition or damage rating at each sampling date.

- o Temperature, weather conditions and rainfall throughout the test.
- o Larval counts and mean damage ratings for each treatment should be compared by use of a multiple range test.

References

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White Grubs

Certain white grubs, the larval stages of scarabaeid beetles, feed on the roots of turfgrasses, often causing severe damage to them. White grubs are found as turf pests throughout the entire United States and include several different genera and many different species. Suppression of many of these pests may be brought about through the use of *Bacillus popilliae*, *B. lentimorbus* or various strains of each (Tashiro 1973). Use of this method has the advantage of permanence in that once introduced into the soil, the bacterial spores remain inactive but viable for years until ingested by grubs. These become infected and produce more spores to add to the soil inoculum. Under ideal conditions, an equilibrium between pest and pathogen is established in which the numbers of grubs present is maintained near the economic threshold which Tashiro (1973) states as being between 1-5 grubs per square foot, depending on the locality in the United States.

Crop and Location of Tests: -- Most, if not all grasses are subject to damage by white grubs. The species of grasses tested should be dictated by the grass species being damaged by grubs in a given locality.

Location of tests is most important, since evaluation may be desirable over a period of years. Plots should thus be placed in areas where land uses are to remain constant for at least 3-5 years. It is also desirable to establish plots in a variety of localities so that as many environmental factors as possible, i.e., soil type, soil pH, soil temperatures, etc., may be examined as to their influence on the host-pathogen interaction. The sites selected should be protected from other insecticide applications throughout the test period.

Plot Size and Design: -- Plots of ca. 4 x 4 to 6 x 6 m have been utilized successfully for determining efficacy of various strains of *Bacillus popilliae* and *B. lentimorbus*. 3 or 4 replications of each treatment should be established and standard recommended chemical control plots as well as untreated check plots should be included. Plots should be well-separated if possible since infected beetles are known to move and spread the inoculum laterally at rates of over 1.5 m in 3 years and have in fact been shown to move at rates of 1 foot/day under extreme experimental conditions. If the demonstration of efficacy for one season only is desired, this factor is of less importance and plots may be placed closer together.

Application and Equipment: -- Application is achieved simply by placing measured quantities of spore-carrier mixtures at uniformly spaced points over the surface of the plots. One manufacturer recommends that these points be on 4-foot centers. It is important to thus provide centers of heavy concentrations of spores so that grubs feeding in those areas will consume lethal quantities of spores and later die. Their cadavers then release spores and form new epicenters away from the original release site. Natural soil mixing by both grubs and other soil invertebrates tends to distribute spores even further until they are distributed throughout the entire plot at infectious levels.

The rate of spread and thus the rate of reduction of grubs is dependent upon the initial dosages applied and the grub population density. Since complete coverage of a plot with high rates would be economically unfeasible, although rapid results might be obtained, it is suggested that application using field control methods be followed in field test plots for determining efficacy.

Sampling: -- White grub counts are made from 0.1 m^2 soil samples randomly located within plots and excavated to a depth appropriate for beetle activity under the conditions at the time of sampling. Grubs move vertically within the soil as moisture and temperature conditions change. Notes on stand condition should accompany larval counts. Counts should be made every 2 weeks with sufficient samples being taken to provide statistically analyzable data. Larvae collected should be returned to the laboratory for microscopic examination of hemolymph to confirm infection. Grubs should be counted by developmental stages as well as in total because of the correlation between species, time of year, susceptibility and lethality as discussed by Tashiro (1969).

Analysis and Reporting of Data: -- The data should be analyzed through multiple range tests for comparisons of means if possible. All environmental conditions should be accurately and faithfully recorded as stated above. Weather conditions during the entire test period should be recorded and summarized for possible correlation with efficacy data.

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FOREST, SHADE AND ORNAMENTAL TREES

The lepidopterous defoliators and sawflies are among the most serious pests of forests and much of our knowledge of insect population dynamics is based on investigations of these species. Quantitative studies in the field and laboratory have shown that viruses do cause epizootic diseases in a number of these species. Among those important forest pests known to be infected by baculoviruses are the gypsy moth, *Lymantria dispar*; spruce budworm, *Choristoneura fumiferana*; Douglas fir tussock moth, *Orgyia pseudotsugata*; tent caterpillars, *Malacosoma* spp.; and the sawflies (Stairs 1972).

The use of *Bacillus thuringiensis* against forest pests has been adequately reviewed by Harper (1974). This review covers the above-mentioned pest species in addition to the spring and fall cankerworms and others.

Efficacy in pesticide usage can be measured in two general categories, crop protection and pest population reduction. These categories are generally used in reference to the same treatment year. Usually little or no inference is made to longer-term effects. Crop protection concepts as related to forest insect control generally means foliage protection. In some cases, there are several levels of foliage protection that could be acceptable and these depend upon the management objectives. Thus, in determining efficacy the amount of permissible defoliation should be given, and its basis related to a specific management objective.

When pest reduction is used in the evaluation it is usually expressed in percent mortality. In the case of forest pests the residual population levels might be more meaningful and possibly the quality of the population. The "desired effects" might depend on the users' or managers' preferred needs. As an example, regulatory officials might desire virtual elimination of the pest, but those in recreational areas would not need the population level reduced so severely. Consequently, there can be several levels of efficacy depending upon particular user's needs.

The following discussion considers some general guidelines for determining efficacy of a microbial material applied to a forest pest species.

Plot Size: -- Usually plot size is determined by whether the test is classified as an experimental or a pilot study. With the experimental test the microbial material may be applied by either ground or air equipment. If applied by ground, the minimum size should be no less than 0.041 ha, and when applied by helicopter or fixed-wing the minimum plot sizes 2.02 and 10.1 ha, respectively, are desirable. Under the operational conditions of the pilot tests the objectives are to establish experimental use and to determine operational feasibility. Using ground and aerial equipment, 4.04 and 4.4 ha, respectively, are preferred for the pilot test. Usually a minimum of 3-4 replications are considered for each treatment in the experimental test. However, in pilot testing a minimum number of replications for each treatment should be established to yield statistically significant results, and if possible, such tests should generally be conducted in more than one geographical area.

Sampling and Evaluation: -- In evaluating the efficacy of microbial insecticides applied to forests, general foliage protection and population reduction are usually considered. When foliage protection is considered in evaluating the efficacy of a microbial insecticde, the net defoliation and total defoliation may be estimated and/or the relative level of defoliation of a particular tree species may be used. Population reduction, on the other hand, may be estimated by determining the mortality due to each treatment, the residual population and its relationship to levels requiring retreatment or an arbitrary residual number, the effect of the treatment as related to the mortality and the residual level of the pest population in the year following treatment, and the reduction of the pest population to acceptable lower levels as related to biological, economic and/or esthetic concepts.

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The following table lists some defoliating insect pests that attack forests, shade trees and some ornamentals, and have been subjected to field treatment with microbial insecticides.

Common Name	Scientific Name	Hosts
A looper	*Lambdina athasaria athasaria* (Walker)	Eastern hemlock
Bagworm	*Thyridopteryx ephemeraeformis* (Haw.)	Maple, pines, wild cherry, poplar, oaks, junipers, arborvitae
Black-headed budworm	*Acleris variana* (Fern)	Western Hemlock

California oakworm	*Phryganidia californica* Packard	Coast live oaks
Douglas fir tussock moth	*Orgyia pseudotsugata* (McDonnough)	Douglas fir, grand fir
European pine sawfly	*Neodiprion sertifer* (Geoffr.)	Scotch pine, pine spp.
Fall cankerworm	*Alsophila pometaria* (Harris)	Elm, hickory, linden, maple, ash, beech, boxelder, basswood, cherry, oaks
Fall webworm	*Hyphantria cunea* (Drury)	Persimmon, pecan, sourwood, black walnut, hickory, cherry, sycamore, crab apple, sweet gum
Forest tent caterpillar	*Malacosoma disstria* (Hbn)	Aspen, water tupelo, hard maple, gums, oaks
Great basin tent caterpillar	*Malacosoma fragile incurva* (Smith)	Cottonwood, willow
Gypsy moth	*Lymantria dispar* (L.)	Oaks, basswood, birch, willow, hemlock, pine, cedar, spruce
Hemlock looper	*Lambdina fiscellaria* (Guenee)	Arborvitae, hemlock, junipers
Lodgepole needle miner	*Recurvaria milleri* (Busck)	Lodgepole pine
Mimosa webworm	*Homadanla albizziae*	Honey locust
Omnivorous leaf roller	*Platynota stuctana* (Walsingham)	Many ornamentals, Euonymus
Orangestriped oakworm	*Anisota serratoria* (Smith)	Red oak
Pine butterfly	*Neophasia menapla* F. and F.	Ponderosa pine
Pitch pine looper	*Lambdina arthasaria pellucidaria* (G and B)	Pitch pine
Redheaded pine sawfly	*Neodiprion lecontei* (Fitch)	Shortleaf, loblolly, lingleaf, slash pines

Saddled prominent	*Heterocampa guttivitta* (Walker)	Beech, sugar maple
Spring cankerworm	*Paleacrita vernata* (Peck)	White oak, red oak, black cherry
Spruce budworm	*Choristoneura fumiferana* (Clemens)	Balsam fir, red and white spruce, larch, pine, hemlocks
Walnut caterpillar	*Datana integrrima*	Walnut
Western hemlock looper	*Lambdina fiscellaria lugubrosa* (Hulst)	Western hemlock
Western tent caterpillar	*Malacosoma californicum*	Hawthorn

A looper, *Lambdina athasaria athasaria* (Walker)

Plot Design: -- Usually hemlocks occur in greatest abundance in moist areas in draws or along water courses; consequently, spray areas may be irregular in size and slope. Treated plots should be at least 20.3 ha for aerial application, and each treatment replicated 3 times. Control areas should be in the same general area and separated by sufficient distance to avoid drift.

Application Equipment: -- A variety of aerial type equipment may be used. Often the Bell 205 helicopter is employed applying the material through 8003 flat fan nozzles, at a swath width of 60 m.

Timing and Frequency of Application: -- Application should be made during the early larval instars.

Sampling and Evaluation: -- Two methods may be used to obtain efficacy data: direct counts of larvae on branch tips and direct counts of larvae knocked down into screens set under trees.

Twenty separate trees can be selected in each of the treatment replicates. The criterion for the selection of an individual sample tree is that there is positive evidence of larval activity prior to treatment. A branch tip sample, 38-46 cm long can be removed from each tree. The branch sample can then be shaken vigorously over a drop cloth and the number of larvae determined with observations made concerning their condition. Larval counts should be made 1 day prior to treatment at intervals of 1, 2, 4, 7 and 14 days after treatment.

Larvae numbers may also be obtained by the amount of droppings onto muslin-covered frames 59.2 x 59.2 cm (3054.64 cm^2). Larval counts again should be made 1 day prior to treatment, and at intervals of 1, 2, 4, 7, 14 and 21 days postspray.

References

Cameron, E. A., and V. C. Mastro. 1975. Control of a looper, *Lambdina athasaria athasaria*, on hemlock with three chemical insecticides. *J. Econ. Entomol.* 68(6): 800-802.

Bagworm, *Thyridopteryx ephemeraeformis* (Haworth)

Plot Design: -- When ground applications are to be made, 50 trees, 1.5 m 2.5 m tall should be selected in alternate rows to minimize spray contamination from drift. Three - 5 replicates should be treated for each dose. In some cases 2 trees may serve for each treatment and each treatment should be replicated 5 or more times. They should be adequately spaced to prevent any unnecessary drift. Individual ornamental trees about 1.5 m high, have also been treated. Each treatment of this type should be replicated a sufficient number of times to obtain acceptable statistical evaluation. Control groups should be treated in the same manner.

Application Equipment: -- A variety of equipment is used for ground application ranging from 10-12 liter hand-operated compression sprayers to solo-mist blowers. Application is usually made to run-off.

Timing and Frequency of Application: -- Treatments should be made after all the eggs have hatched in the bags. Applications made one week after egg hatch have been proven to be satisfactory.

Sampling and Evaluation: -- The postspray larval determinations may be made by counting the number of living larvae on 25 cm terminals of the previous year. Evaluation should be made following each spray date if multi-applications are investigated. 7-day posttreatment samples of 100 bags each may be taken from each treated tree and untreated tree, and the number of dead larvae determined. Foliage may also be removed and weighed to evaluate the extent of bagworm damage.

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Kearby, W. H., D. L. Hostetter, and C. M. Ignoffo. 1972. Laboratory and field evaluations of *Bacillus thuringiensis* for control of bagworms. J. Econ. Entomol. 65: 477-480.

Western Tent Caterpillar, *Malacosoma californicum*; Black-Headed Budworm, *Acleris variana* (Fern)

Plot Design: -- When individual trees are treated a minimum of 5 should be sprayed with each material and each treatment should be replicated 2-3 times; an untreated group should also contain the same number of trees.

Application Equipment: -- Applications are made with a variety of sprayers and dusters and include back-pack mist blowers and pressurized hand sprayers.

Timing and Frequency of Application: -- Microbial materials should be applied during the early instars and when sufficient foliage is present.

Sampling and Evaluation: -- When evaluating treatments for tent caterpillars pre-and postspray population estimations should be undertaken. Tents may be selected at random from each treated tree and the number of dead larvae determined.

Pre-and postspray population densities should be determined for the budworm. Samples of an 18" branch tip, taken from the top third of the crown with a sectional pole pruner may be used to estimate the population.

California Oakworm *Phryganidia californica* Packard

Plot Design: -- When ground application is used, individual trees or groups of trees may be treated with the selected material. When the individual tree method is used, an adequate number should be employed for each treatment with an appropriate replication. Thus, plot selection method could also apply to groups of trees.

Application Equipment: -- The various materials may be applied with conventional high pressure tree sprayer, single gun line pressure equipment and motorized knapsack dusters.

Timing and Frequency of Application: -- The applications should be made during the early 3rd larval instar, at which stage insignificant damage is done. A single application is usually made, and applied to the point of runoff.

Sampling and Evaluation: -- 25 shoots on each tree should be sampled at random, and the number of eggs and larvae noted. Each shoot will contain 10 or 11 leaves on an average, and thus, more than 250 leaves per tree will be sampled. The average number of larvae per 25 shoots may be used for comparing treatment trees with untreated trees.

Reference

Pinnock, D. E., and J. E. Milstead. 1971. Control of the California oakworm with *Bacillus thuringiensis* preparations. *J. Econ. Entomol.* 64: 510-513.

Douglas Fir Tussock Moth, *Orgyia pseudosugata* (McDonough)

Plot Design: -- Plots may be 8.1-60.7 ha in size for aerial application. Thoughout each plot, 25 trees in 5-tree clusters are designated for population density sampling. Sample trees may be open grown Douglas fir at about 12.2-15.2 m in height. The experimental design should be replicated 3 times and plot assignments should be made on a random basis. When tree host and insect populations are initially different, efforts should be made to distribute the treatment plots so as to consider any variation. Control plots should also be included in the evaluation.

Application Equipment: -- A Cessna AG as well as other aerial equipment may be used to apply the formulations using a conventional spray boom equipped with T8010 flat fan tips. Swath spacing of 30.5 m results. A Bell 476 helicopter has also been used to apply the various formulations at the rate of 7.57 l per 0.405 ha using a boom-equipped spray system with T8002 flat fan tips. The helicopter may spray at 72.6 kph at ca 15.2 m above the canopy.

Timing and Frequency: -- Treatments should be applied when the majority of the larvae are in the 2nd and 3rd instar.

Sampling and Evaluation: -- Near the center of each plot some 15 trees can be designated for population density sampling. Population density should be sampled prespray and at 7, 14, 21 and 35 days after treatment.

Sampling may consist of counting larvae and measuring the foliage area on three 46-50 cm branches cut with a sectional pole pruner from the mid-crown level of the 15 sample trees in each plot. Larvae counts should be converted to larvae per 6452 cm^2.

Larvae may also be collected at the sampling time and maintained in containers with food for observations. They may be held at 23-24°C and 40-50% RH for 14 days.

Visual estimates should be taken late in the season (September) on the 15 sampling trees. A 15.2 cm ruler be used to divide the foliated bole of the tree into 6 approximately equal length units. Defoliation in each unit may be given a numerical value of 0 (no defoliation), 50 (≤50% defoliation) or 100 (>50% defoliation). The total for the 6 units may be divided by 6 thus giving a percent defoliation per tree. Individual tree values may then be averaged for the plot.

The surviving population density, percent population reduction, deposit and defoliation data can be subjected to analysis of variance; Tukey's w-procedure at the 95% level can be used to compare the treatment means. An analysis of covariance can be conducted in which time can be a split plot factor, to determine the treatment effects over the duration of the field experiment.

References

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European pine sawfly, *Neodiprion sertifer* (Geoffr.)

Plot Design: For aerial applications, treated blocks may be 2-25.7 ha and separated by a buffer strip of at least 91.4 m wide. Ten trees separated from one another by 27-28 m or more may be selected randomly throughout each treatment block and tagged for sampling. Twenty-21 ha has also been satisfactory for evaluating plantation-type treatments. Four subplots 31 x 92 m may be selected in such plantation blocks. Treatment blocks should be replicated 2-3 times. Control should also be included in the evaluation.

For ground application, plots should consist of at least 10 trees for each treatment, and treatments should be replicated 2-3 times.

Application Equipment: -- A Piper Pawnee 235 aircraft equipped with 8 or 10 flat-fan nozzles with 80015 tips may be used. Each nozzle will deliver 4.73 deciliters per min at 40 lb per 6.45 cm^2 line pressure. Spray boom is canted 30° forward into airstream. The air speed is 161.3 kph.

For ground applications, speed sprayers and 11-12 l pressurized hand sprayers may be used.

Timing and Frequency of Application: -- Material should be applied during early instars.

Sampling and Evaluation: -- When the larger blocks are used, approximately 200 trees should be selected for sampling in each subplot. 1 or 2 sawfly colonies should be examined on each tree, and classified into the following categories: (1) colonies all living, (2) colonies with some dead larvae, and (3) colonies with all dead larvae. Posttreatment determinations should be made on a weekly basis for 3 weeks.

References

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Wallner, W. E. 1968. European pine sawfly control with aircraft application of concentrated insecticides. *J. Econ. Entomol.* 61: 1666-1667.

Fall Cankerworm, *Alsophila pometaria* (Harris)

Plot Design: -- For aerial applications, each treatment should be applied to an area of 16.2-20.2 ha and separated by an untreated buffer strip.

For treatment with ground equipment, individual trees that are infested with the larval stage may be selected.

Application Equipment: -- Applications may be made with a Bell G-2 Model 47-G helicopter fitted with a 9.14 m boom and 59 no. D-3W/23 cores nozzle type.

A 3.785 l knapsack sprayer may be used for ground applications. Each treatment is applied to 3 branches selected at random from the covered branches of each of the trees.

Timing and Frequency of Application: -- One or 2 applications may be made depending on the material being tested. Applications should be made on early instars.

Sampling and Evaluation: -- Evaluation may be based upon larval numbers collected from 3 3.05 x 3.05 polyethylene tarps within each treatment block. Tarp placement should be made at random, and larval collections can be made at 2, 4, 7 and 21 days posttreatment.

Where smaller plots are used, individual trees infested with the larval stage may be selected. Seven to 10 branches of each tree to be treated and which are heavily infested with the fall cankerworm larvae should be selected. Selection of nontreatment trees should be done in a similar manner. On each tree, ca. 1.82 m of the distal portion of the selected branches should be covered with a nylon mesh bag (2.74 x 1.22 m). The open end of the bag is then closed and tied to the branch. Just prior to treatment, each bag is removed, sprays applied, and the bag replaced.

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Mimosa Webworm, *Homadaula albizziae* Clarke; Fall Webworm, *Hyphantria cunea* (Drury); Walnut caterpillar, *Danata integerrina* Grote and Robinson

Plot Design: -- Biological materials may be applied on individual trees, and each treatment replicated 3 or more times. Untreated trees should be treated in a similar fashion.

Application Equipment: -- Ground application may be made with mist blowers or back pack mist blowers. Materials are usually applied to run-off conditions.

Timing and Frequency of Application: -- The first application should be made to larvae during their early instars. A second application may be necessary 7-10 days later.

Sampling and Evaluation: -- Webworms and the walnut caterpillars may be confined in sleeve cages (40-mesh cooper wire screen (15 cm dia x 46 cm long)) placed on each of the trees. Cages are slipped over the end of a branch which has 25-30 undamaged crotches. Each week following applications, 25 larvae are placed in each cage.

English, L. L., and W. Hartstirn. 1962. Systemic insecticide control of some pests of trees and shrubs —— a preliminary report. *Nat. Hist. Survey Div. Biol. Notes.* No. 48: 1-12.

Forest tent caterpillar, *Malacosoma disstria* (Hubner)

Plot Design: -- For aerial treatments, plots should be 4-20 ha and, if possible, separated by an untreated buffer strip. Control plots of equal size should be located near treatment plots to minimize differences in responses that might be due to biotic and climatic factors.

Application and Equipment: -- Applications may be made with a Bell G-2 Model 47-G helicopter fitted with a 9.14 m boom and 50 no. D-3 W/23 cores nozzle-type.

Applications have been made with a Piper Pawnee aircraft fitted with 10 Roto-spin nozzles (5/wing), each of which contained a No. 6 orifice, hollow-core spray nozzle and a plastic, wind-driven propeller which further broke up the emitted spray. Materials are applied from a height of 15 m above the canopy at an air speed of 100 knots. Some materials may be applied at 3785 cm^3/0.405 ha in water-based formulations. Whenever possible, aerial application should be monitored from a spotter plane.

Timing and Frequency of Application: -- Egg hatch and subsequent larval development should be monitored by weekly inspection of egg mass samples. Treatment applications should be timed whenever possible to reach the caterpillars when the majority are in the 2nd and 3rd instar. In normal years, the foliage is ca. 1/2 to 2/3 expanded at this time. Second and 3rd instar larvae will usually consume sufficient foliage to have a high probability of ingesting the stomach-type microbials, yet not so much feeding that average populations will cause severe damage to the tree crowns. In some cases, a second application will be needed.

Sampling and Evaluation: -- Evaluation should be based upon larval numbers collected from 3 3.05 x 3.05 m polyethylene tarps within each treatment block. Tarp placement should be at random, and larval collections should be made at 2, 4, 7 and 21 days posttreatment.

Branches may also be removed from the uppermost crowns of dominant trees. Egg masses may then be counted and recorded as the average number per 1.23 m branch tip. Usually with water tupelo an average of 2.5 egg masses per sample produces a caterpillar population capable of completely defoliating the canopy and understory foliage of a stand by the end of the defoliation period. Samples averaging less than 2.5 egg masses per sample will yield larval populations that can cause partial stripping of the canopy, while numbers greater than 2.5 cause accelerated defoliation.

Efficacy of eath treatment may be monitored by ground observations in each plot beginning within 24 hours posttreatment and continuing at 7-day or greater intervals until all larval feeding activitv has ceased. On each observation date, percentage of canopy defoliation should be estimated for each treatment and control plot. Larvae on branches that are removed from treetops in each treatment area should be examined, and the numbers, size and general condition of these larvae determined.

References

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Wallner, W. E. 1971. Suppression of four hardwood defoliators by helicopter application of concentrate and dilute chemical and biological sprays. *J. Econ. Entomol.* 64: 1487-1490.

Great basin tent caterpillar, *Malacosoma fragile incurva* (Henry Smith)

Plot Design: -- For aerial application the plot size for each treatment can be a 10.1 ha block and should be replicated. The untreated blocks should be separated from the treated blocks by a buffer strip at least 91.4 m wide.

Application Equipment: -- Applications may be made with a helicopter or other conventional air spray equipment.

Sampling and Evaluation: -- Two 0.81 m^2 subplots should be set up in each hectare of the treated and untreated plots. Measurement of treatment effectiveness should be based upon the following: (1) the rate of disease mortality of larvae reared in dacron sleeve cages, and (2) the percent of the colonies containing disease-killed larvae. Groups of 25-50 larvae may be caged on the terminal of a 92 cm branch, located on each of a given number of cottonwood and/or willows in the subplots.

Colonies of the tent caterpillar may be removed from the host trees in each subplot with a pole pruner at 1-3 day intervals after being sprayed. They may then be examined for the presence or absence of disease-killed larvae and the percent of infected colonies. Examination may be continued until 25 days after spraying or until pupation.

References

Stelzer, M. J. 1969. Control of a tent caterpillar, *Malacosoma fragile incurva*, with an aerial application of a nuclear-polyhedrosis virus and *Bacillus thuringiensis*.

Gypsy Moth, *Lymantria dispar* (L.)

Plot Size: -- When microbial insecticides are applied by air, plots should be a minimum of 10.12-20.24 ha and replicated 3 times. Plot size for ground applications may vary, but usually 0.1 ha has been found to be satisfactory. The treatment site should be selected so as to contain at least 50% primary gypsy moth host species. Efforts should be made to obtain reasonable uniformity in the plot stand structure.

Application Equipment: -- Ground application may be made using truck-mounted mist blowers. Adequate spray coverage of 0.1 ha plots is usually achieved using ca. 76.0 l. Aerial application may be made with either helicopter or fixed wing aircraft. As an example, applications to experimental plots have been made with a 450 Grumman Ag Cat equipped with 6 Beecomist nozzles. Application is usually made at a height of 9-18 m above the canopy at a speed of 2.1-2.4 km/min.

Timing and Frequency of Application: -- Usually 1 or 2 applications of the microbial insecticide are made. The initial spray application is made when at least 50% of the egg masses have hatched and when at least 50% leaf expansion has occurred. If a 2nd application is to be made, it is usually applied 5-10 days after the 1st spray.

Sampling and Evaluation: -- Egg mass determinations —— Subplots of 101.2 m^2 size can be selected at random using about 10 10.12 ha treatment plots. Egg mass determination should be made in each subplot prior to spraying. These determinations should be made as thoroughly as possible including fallen limbs, rock piles, loose bark, etc. Postspray determinations should be made after leaf drop. For subplot selection the "prism point" plot selection method may also be used (Wilson and Fontaine 1977).

Burlap bands —— Five or more trees in each subplot should be banded with burlap (a 15 cm burlap strip wrapped twice around the trunk 1.5 m above ground, flap may be made by cutting into the burlap strip every 8 cm). Larval density is then determined by counting larvae under the burlap on a biweekly basis until pupation.

Terminal branch determination —— Terminal counts may also be made. Select 25 59 cm terminals in each subplot and determine the number of larvae on each of the terminals.

Defoliation estimates —— Defoliation estimates should be made in each 101.2 m^2 subplot immediately prior to spraying, and again at the completion of larval feeding. The estimates may either be made from aerial photography or ground observations. Five primary host trees can be selected or the entire subplot may be estimated for defoliation. The damage level by tree or subplot may be rated by the following scheme: 0-19, 20-39, 40-59, 60-79, 80-100% defoliation.

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Yendol, W. G., R. A. Hamlen, and F. B. Lewis. 1973. Evaluation of *Bacillus thuringiensis* for gypsy moth suppression. *J. Econ. Entomol.* 66: 183-186.

Hemlock looper, *Lambdina fiscellaria* (Guenee)

Plot Design: -- Trees or ornamentals planted in rows may be treated by ground application. As an example, 6 rows containing approximately 150 plants may serve for each treatment.

Application Equipment: -- Conventional, high-pressure, high-volume hydraulic sprayer equipped with a spray gun may be used.

Timing and Frequency of Application: -- Single applications are usually made during the 5th instar.

Sampling and Evaluation: -- The number of larvae per individual plant is usually determined. An appropriate statistical number of plants should be used for sampling each treatment. Evaluation should be made on the 10th-20th posttreatment day.

References

Kerr, T. W. 1971. Control of the hemlock looper. *J. Econ. Entomol.* 64: 1552.

Lodgepole Needle Miner, *Recurvia milleri* (Busck)

Plot Design: -- Individual trees may be used for testing each material and specific concentration. A plot may consist of 3 trees and/or tree group. Plots should be replicated 2-3 times.

Application Equipment: -- Ground applications may be made with a truck-mounted mist blower or back pack mist blowers. Attempts should be made to obtain thorough coverage.

Timing and Frequency of Application: -- Application should be directed against the 1st instar larvae migrating to their 1st needles after eclosion.

Sampling and Evaluation: -- 10 twigs with 5 whorls of needles should be taken from each treated tree to ascertain the larval population. Larval counts are then made and the treatments compared with the controls.

Prespray sampling should be undertaken to determine the population.

References

Struble, G. R. 1965. Field test of *Bacillus thuringiensis* (Berliner) to control lodgepole needle miner. *J. Econ. Entomol.* 58: 1005-1006.

Omnivorous Leaf Roller, *Platynota stultana* (Walsingham)

Plot Design: -- Individual trees or ornamental bushes may be treated individually. Each treatment should be replicated with sufficient numbers (3-4 each).

Application Equipment: -- Full coverage sprays may be applied with small aerosol units.

Timing and Frequency of Application: -- Usually single applications have been done.

Sampling and Evaluation: -- The total number of live larvae on each treated plant should be determined at 10 days, 10 and 20 weeks posttreatment.

References

Campbell, R. L., and B. G. Ward, Jr. 1971. Insecticides tested against *Platynota stultana* on Euonymus. *J. Econ. Entomol.* 64: 1556.

Orangestriped oakworm, *Anisota senatoria* (J. E. Smith)

Plot Design: -- With small-scale ground applications one tree may serve to test each treatment and/or concentration, and should be replicated 3 or more times. Care should be taken to have buffer trees between treated and untreated trees.

Application Equipment: -- Materials may be applied with a hydraulic sprayer at 400 lb/m^2.

Timing and Frequency of Application: -- Attempts should be made to apply microbial materials during the early instars.

Sampling and Evaluation: -- Since *Anisota senatoria* are gregarious, leaf counts should be made on branches only where the insect occurs. These branches may be tagged and pre- and postspray leaf counts made. Twenty leaves per tree are sampled and the number of larvae occurring on each leaf can be recorded. Counts should be taken at 1, 2 and 4 days posttreatment.

Two 0.835 m^2 cloth trays may also be placed under each tree. The dead and moribund larvae should then be determined.

References

Kaya, H. K. 1973. Laboratory and field evaluation of *Bacillus thuringiensis* var. *alesti* for control of the orangestriped oakworm. *J. Econ. Entomol.* 67: 390-392.

Pine Butterfly, *Neophasia menapia* F. and F.

Plot Design: -- For aerial application experimental blocks should be 16.2 ha (20 x 20 chains). Each dosage should be applied to 3 randomly selected blocks. 3 blocks should be left untreated to serve as control blocks.

Application Equipment: -- Conventional aerial application equipment may be used.

Timing and Frequency of Application: -- Applications should be timed so as to treat early instar larvae.

Sampling and Evaluation: -- 5 sample plots of 10 trees each may be selected in each block. Prespray and postspray larval counts can be made by removing 6 branches ca. 12.7 cm from each sample tree during each sample period. Prespray larval counts should be made 24 hours prior to spraying. Postspray counts should then be made 4, 8 and 12 days later. Prespray and postspray larval densities can be expressed as the number of larvae per lineal 2.54 cm of foliated branch.

Each plot may be aerially photographed with color IR late July to August to measure the degree of defoliation.

Calculate the number of larvae per 2.54 cm of foliated branch.

References

Cessla, W. M., and J. E. Dewey. 1973. Control of pine butterfly in western Montana with aerial applications of mexacarbate and *Bacillus thuringiensis*. Ann. Meeting Western Forest Pest. Comm. 64th West. Forestry Conf., San Jose, CA, December 5, 1973.

Pitch Pine Looper, *Lambdina athasaria pellucidaria* (G & R)

Plot Design: -- Plots should be a minimum 10.1 ha for aerial application and should be replicated 2-3 times Distances between plots should range between 92-183 m.

Application Equipment: -- The microbial insecticide may be delivered with a Bell G-3 helicopter which travels about 97 kph, 3.5 m above the canopy, with a swath about 31.1 m. Eight D-4 tip nozzles operated at 40 lb. per 6.452 cm^2 have been used.

Timing and Frequency of Application: -- A single application is usually made during the early instars when sufficient foliage is present.

Sampling and Evaluation: -- Four transects per treatment plot should be established perpendicular to the direction of the spray operation. Larvae are then collected randomly from selected trees along each transect. Five trees should be used for every 2.02 ha sampled in each treatment. Samples 46 cm long should be cut from terminal and lateral branches at a height of 92-299 cm. Samples should not be selected within 15.2 m of the edge of the treatment block. The 46 cm samples are beaten over a 76 x 76 cm piece of plastic until no further loopers fall off the sample. Prespray determinations should be made and comparative evaluations should be made 3 or more days postspray.

ANOVA, t-tests and proportional comparisons can be used to determine significance.

References

Sorenson, A., and P. Barbosa. 1975. Effectiveness of carbaryl and thuricide 16-B on a population of larval *Lambdina athasaria pellucidaria*. *J. Econ. Entomol.* 68: 561-562.

Redheaded Pine Sawfly, *Neodiprion lecontei* (Fitch)

Plot Design: -- Aerial applications should be made on 10.12-20.24 ha blocks. Ground applications (mist blowers) may be made on 2.02 ha blocks.

Application Equipment: -- Application may be made with fixed wing aircraft at 145.2 kph, ca 15.2 m above tree tops and a 30.5 m swath wide. Such a spray system produced 22.7 kg per 6.452 cm^2 of pressure with 8 no. 80015 flat fan spray nozzles.

With ground applications, mist blowers or smaller back pack equipment may be used.

Timing and Frequency of Application: -- The first application should be made when larvae are in the 2nd to 3rd instar.

Sampling and Evaluation: -- Before treatment, 100 larval colonies can be selected randomly on the trees to be treated. On the day before treatment larvae be counted in each tagged colony or estimated by tens (to minimize disturbance) if the colony is large. Living larvae should then be counted in the same colonies at 7, 14 and 21 days posttreatment.

References

Fowler, R. F., I. Miller, and L. F. Wilson. 1973. Aerial and ground applications of malathion for control of the redheaded pine sawfly. *J. Econ. Entomol.* 66: 288.

Wallner, W. E. 1968. European pine sawfly control with aircraft application of concentrate insecticidal sprays. *J. Econ. Entomol.* 61: 1666-1667.

Spring Cankerworm, *Paleacrita vernata* (Peck) and Saddled Prominent, *Heterocampa guttivitta* (Walker)

Plot Design: -- Each treatment may be applied to an area of 16-20 ha separated by an untreated buffer strip.

Application Equipment: -- Applications may be made with a Bell G-2 Model 47-G helicopter fitted with a 9.14 m boom and 59 no. D-3 W/23 cores nozzle-type.

Timing and Frequency of Application: -- Initial applications should be made when sufficient foliage is present, and if possible, when majority of larvae are in the 2nd and early 3rd instar.

Sampling and Evaluation: -- Evaluation may be based upon larval numbers collected from 3 3.05 x 3.05 m polyethylene tarps within each treatment block. Tarp placement should be at random, and larval collections should be made at 2, 4, 7 and 21 days posttreatment. Comparison should be made with untreated blocks.

References

Wallner, W. E. 1971. Suppression of four hardwood defoliators by helicopter application of concentrate and dilute chemical and biological sprays. *J. Econ. Entomol.* 64: 1487-1490.

Spruce Budworm, *Choristoneura fumiferana* (Chem.)

Plot Size: -- Usually when aerial applicatons are made, 10-20 ha are used and each treatment is replicated at least 3 times. Treatment blocks should contain at least 0.4 km between each to act as a buffer zone. In the case of ground applications, 1 ha blocks be used. Care should be taken to select uniformity in blocks with regard to forest composition. In some situations hand spraying of individual trees may be desirable and at least 30 or more trees be considered for each treatment.

Application Equipment: -- Usually sprays are applied in the early morning and/or late evening. In the case of Morris and Armstrong (1975), a Cessna Agtruck aircraft equipped with 4 AU 3000 Micronair emission units calibrated to deliver droplets in the 50-100 μ dia. range was used. Diamond (1972) used a helicopter to apply the microbial insecticide.

Timing and Frequency of Application: -- Usually 1 or 2 applications are made and this may depend on the type of material being tested. Spraying is usually done in the morning or evening and when the budworm development reaches 60% in the 4th instar, 30% in the 3rd instar and the remainder in the 2nd and 5th instars.

Sampling and Evaluation: -- Within each plot, 20 codominant fir trees should be selected for sampling. If the stand is mixed spruce and balsam, 25 spruce and 25 balsam should be used. These trees should be selected at random across the plot, but no closer than 30 m to the plot boundaries.

Population levels of the spruce budworm can be determined by making counts of larvae and/or pupae on 2 38 cm branch terminals for 25 trees of [illegible] erminations have been made by using 46 cm (18.1") of the branch tip of white spruce and balsam fir. Prespray and postspray collections should be made in both treatment and control plots. Posttreatment samples should be made 6-10 days after spraying and then again at 2-3 weeks postspray which can be timed with the termination of larval feeding (Diamond 1972). Morris (1975) on the other hand, has used the 30 postspray day period as a final and probably the most important postspray sample, the peak of pupation. Approximately 200-400 pupae should be collected from each plot and reared for emergence. The actual residual population should be based on the live pupae which is based on emergence. The corrected population reduction (i.e., reduction due to treatment) may then be calculated on this residual population.

Defoliation estimates should be made after cessation of feeding. The following method is proposed by Diamond (1972): ten 38 cm branches are pruned from each 25-sample trees per plot. The current year's shoots should be examined and classified as either destroyed (the entire year's shoot is missing), damaged (shoot with apex intact and a bud formed to a healthy fully-needled shoot with portions of 2 or 3 needles missing), or undamaged (no signs of feeding). This can be converted to a single value. Although somewhat time-consuming, the "Fettes' branch examination method" may be used.

	Destroyed	Damaged	Undamaged
Sum of buds from 5 branches in each category	76	207	18
Percent of buds in each category	25%	69%	6%
Sum: destroyed % x 2, damaged % x 1, undamaged % x 0	(25 x 2) 50	+ (69 x 1) + 69	+ (6 x 0) + 0
Damage rating =		119	

Obviously, a tree with extreme damage would have 100% of its shoots in the destroyed category and a damage rating calculated at 200. Conversely, a completely undamaged tree would calculate to a 0 damage rating. Other trees would fall somewhere between on this 200-point scale.

Unusual defoliation estimates may also be used in comparing treatments with non treated plots. The 25 trees in each plot may be rated using the scale 0-19, 20-39, 40-59, 60-79, 80-100% defoliation. Visual estimates may be made of the upper and lower crown, and the mean defoliation calculated for the plot and treatments.

In some cases it is advisable to reassess 1 year after treatment to determine any long-term effects on defoliation. The amount of defoliation that stands can tolerate is perhaps still in question. Morris and Armstrong (1975) suggest that less than 50% defoliation of the current year's growth is generally considered to be not seriously injurious to tree growth and health. Using the damage conversion method Diamond (1972) suggests that defoliation ratings of 100 or less are tolerable. For other information concerning evaluation of microbials against the spruce budworm, consult Morris and Hildebrand (1974) and Morris (1977).

References

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Western hemlock looper, *Lambdina fiscellaria lugubrosa* (Hulst)

Plot Design: -- For aerial application the treatment blocks may be 10 or more ha of infested forest. Each treatment block should then be replicated 3 times. An untreated area of the same size should also be developed. Treatment blocks should contain at least 6-7 sampling plots, which can be located on cardinal directional lines constructed through the block. Sampling plots should be located at 137-146 m intervals along lines. Each sampling plot should consist of 3 codominant hemlocks.

Application Equipment: -- The material may be applied with a Bell G-2 helicopter equipped with a 9.1 m boom and 18 no. 4664 Tee Jet spray nozzles, delivered at the rate of 7.57 l per 0.405 ha. Mass median diameter (M.M.D.) should be about 160-170 microns.

Timing and Frequency of Application: -- Spraying should be timed with the peak number of 3rd instar larvae.

Sampling and Evaluation: -- Mortality should be assessed by counting and collecting dead larvae found at intervals in ground trays. Ground trays with an inside area of 0.19 m^2 have been found to be adequate. The ground trays are positioned under the hemlocks in each sampling plot. Ground trays should be emptied prior to spraying, and then examined every 2 days until 20 days after spraying.

Quantitative estimates of larval mortality should be obtained from periodic counts of larvae on foliage samples collected with a pole pruner equipped with a basket. At each sampling, 5 46 cm branch tips can be clipped from each plot tree; the total larvae on the 15 branch tips from the 3 trees represent the population estimate for the plot. Trees may be sampled 3 to 4 days before spraying and at 3 day intervals postspray. Estimates of the populations can then be made by comparing the number of larvae on 15 branch tips before test-area spraying, and 19 days after spraying. Using data collected in the spray area, a regression analysis may be performed to determine the significance of downward population trends.

The frass-drop method may also be used to assess mortality. Its use for estimating populations is based on determining: (1) the amount of frass falling on a tray during a known period of time, and (2) the amount of frass produced by an average larva during the same period.

STORED PRODUCTS

Bacteria and baculoviruses appear to have potential for controlling certain species of stored-product insects. Although studies have been reported of the pathogen susceptibility of numerous species that infest stored products, serious attempts at pest control have been limited to three species of Lepidoptera: the Indian meal moth, *Plodia interpunctella* (Hubner); the almond moth, *Cadra cautella* (Walker); and the tobacco moth, *Ephestia elutella* (Hubner). These studies have dealt with infestations in dried fruits, nuts, cereal grains and tobacco in very restricted commodity storage situations. More limited studies have been made on the control of these pests and the Mediterranean flour moth, *Anagasta kuehniella* (Zeller) in flour, and the greater wax moth, *Galleria mellonella* (L.) and the lesser wax moth, *Achroia grisella* (F.) in beeswax and beecomb. The existing methodology will require modification and extension as pathogen uses are proposed in other commodities and storage situations, and when promising pathogens of species of stored-product infesting Coleoptera are proposed as control measures. The literature on test methods for chemical insecticide evaluation may be useful for that purpose.

The general considerations and steps in product development that introduce this report are generally applicable to stored commodities. Only specialized procedures that are unique to tests on stored commodities are discussed in this section. The methods presented are not to be considered exclusive of other valid methods that have been used or that will be developed in the future.

Unlike control measures for most phytophagous pest species, residual insecticides may be used as preventive treatments on stored commodities. They are usually applied as the commodity is moved into storage or before infestation is apparent. Applications of pathogens made after infestations are apparently effective only against subsequent generations of the pest. In such cases other means of immediate population reduction such as fumigation may be used prior to application of the pathogen. Because residual insecticides are used primarily as preventive treatments, the frequently used parameters of efficacy such as population reduction are not always applicable. Furthermore, because the insecticides are usually intended to provide protection for several months or years, efficacy must be evaluated over a long period of time.

Most stored commodities are subject to infestation by several species of Lepidoptera and Coleoptera. Insect pathogens, because of their relatively narrow spectrum of activity, will individually be unsuitable for controlling all of the pest species that may be present in the commodity at one time. They will be useful, however, in pest control programs in conbination with other insecticides and pest control practices, and for controlling certain pests (particularly species of Lepidoptera) whose unique patterns of infestation necessitate the use of additional protective measures. Compatibility with other insecticides and with fumigants must be assured if they are used together.

Because insects are considered to be contaminants as well as destroyers in stored foods, the objective of all control measures should be complete control or prevention. Presence of a detectable infestation would indicate unsatisfactory control. It should be recognized, however, that the best available control measures may fall short of this objective.

Harvested agricultural commodities that are subject to insect infestation are stored under conditions that can be readily simulated in the laboratory. Therefore, in contrast to pest control on growing plants, extensive testing of stored-product pest control materials can be done in the laboratory on a relatively small scale, using laboratory-reared insects. Pilot and commercial scale tests are used to confirm the results of the laboratory studies and to make any necessary adjustments in dose or application procedure that may be required as a result of the larger storage units and the wild insect populations.

Certain commodity quality factors including variety, moisture content, broken particles, and foreign material are known to affect the infestability of commodities and the performance of insecticides. These factors should be considered when selecting commodities for use in tests, and their interaction with the treatment should be evaluated in the earlier stages of testing.

Insect Rearing

The extensive reliance on laboratory testing makes the maintenance of healthy, vigorous, genetically-stable colonies of insects mandatory. Several diets have been successfully used for rearing species of stored-product infesting Lepidoptera. Most make use of mixtures of ground cereal grains fortified with yeast, glycerol and honey, and some require the addition of water and fungistatic agents. Sterilization, usually be autoclaving of the cereal grain components is necessary to exclude storage fungi and any insect pathogens that may be present on these commodities as a result of prior insect infestation during storage. The insect eggs are usually surface-sterilized with dilute solutions of formaldehyde or other suitable materials to reduce the transfer of pathogenic microorganisms from generation to generation. Other laboratory practices designed to assure a vigorous and genetically stable colony should be employed in order to obtain laboratory results that will be reproducible under commercial commodity storage conditions.

References

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Bioassay

The larvae of species of stored-product infesting Lepidoptera develop faster and with less mortality in fortified diets than in natural food products. Therefore, bioassays that make use of these fortified diets appear to provide greater precision with fewer test insects in a shorter period of time. Conventional analytical techniques such as probit analysis are satisfactory for estimating LC_{50} values and the slopes of dose-mortality relationships. Low slopes and variation in slope and LC_{50} estimates between assays necessitate ample replication and vigilance to assure parallelism, particularly in comparative studies and standardization tests.

A bioassay that has been useful for evaluating the efficacy of *Bacillus thuringiensis* against Indian meal moth and almond moth larvae is presented in Exhibit 3. This assay has also been used for standardizing a formulation of Indian meal moth granulosis virus. It probably will be useful for the tobacco moth and other related species.

References

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Laboratory Testing

Purpose and Scope: -- The initial stages of laboratory testing may involve very small samples of insect diet or commodity (10-200 g) treated with suspensions of crude preparations of pathogens. Eventually, however, tests are usually made in 1-20 kg samples of commodity treated with formulated products. These latter tests involving formulated pathogen products may be used to obtain estimates of minimum effective doses, to determine the longevity of effective insect control, to determine the stability of the treatments under simulated storage conditions, to determine compatibility with other pest control treatments such as fumigants, to compare alternative formulations such as wettable powders, dusts, baits, etc., to study interactions with nonsusceptible pest species, and in some cases to obtain preliminary data regarding the efficacy of various application techniques. The small size of the test units facilitates testing numerous dose rates.

Design and Analysis: -- A minimum of 3 replications are typically used in these tests. More replications are sometimes needed when very small numbers (less than 50) are tested in each experimental unit. Standard statistical procedures such as analysis of variance and multiple range tests are useful for interpreting differences.

Application: -- Because these tests are conducted primarily to evaluate the product rather than the application technology, application can be made by any available means that will assure thorough coverage. Large volumes of diluent or carrier material may be used if provisions are made for drying the commodity to prevent spoilage. For example, in testing on small samples of cereal grains the insecticides have been suspended in water for application at the rate of 21 ml/kg. The suspension was poured into a jar containing the grain sample and the jar was tumbled or shaken until all free moisture was absorbed by the grain and coverage appeared uniform. Dried fruits have been dipped in the suspension, and spraying has been used on tobacco leaves and on nuts. For some commodities commercial treatment procedures may be used to apply the insecticide to larger quantities of the commodity. Small samples of the treated commodity can be held in appropriate containers in the laboratory for determining efficacy.

Evaluation: -- After treatment the commodity samples are held under laboratory conditions in containers that will confine insects, and are subjected to infestation with insect eggs or neonate larvae. Reduction of larval survival of adult emergence serve as a measure of efficacy. The treated samples may be infested immediately after application or they may be subjected to other treatments such as long-term storage in simulated or actual storage environments, or application of other pest control products or processes prior to infestation. The samples may also be held in containers which simulate actual storage units such as vertical columns for grain, commercial packages for dried fruits, or small bundles for tobacco.

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Pilot-Scale Testing

Purpose and Scope: -- Pilot-scale testing may be considered intermediate level testing to be used between laboratory and field testing in instances where (1) field uses are of such magnitude as to make extrapolation from laboratory results questionable, (2) resources are inadequate to enable immediate testing on a commercial scale, or (3) an experimental use permit is not available. The use of pilot scale tests will generally reduce the number of doses that will need to be tested on a commercial scale.

Pilot-scale tests are usually made under actual storage conditions (ambient) using a limited number of standard commodity storage units, or numerous miniature simulated storage units. The test period should coincide with a typical commodity storage period. The temperature and moisture content of the commodity should be monitored during the test. Insects should be permitted free access to and from the test units and the units should generally be of sufficient size to accommodate the normal behavioral patterns of the test insects. However, pilot-scale tests need not rely exclusively on natural insect infestation. Insect infestation can be supplemented in all of the individual bins or test units or in the warehouse in which the test is conducted to the extent necessary to assure heavy infestation of the untreated controls. At least 3 doses should be tested (the expected minimum effective dose plus one higher and one lower dose) to assure an accurate determination of the minimum effective dose.

Design and Analysis: -- Tests should be adequately replicated (usually 3-5 replications are used) and should include untreated control units to accurately evaluate the efficacy of the treatments. Randomized block design is satisfactory, although other designs may be preferred under some circumstances. Standard statistical procedures such as analysis of variance and multiple range tests are useful for interpreting the data.

Application: -- When possible, the pathogen should be applied using techniques that are the same as or closely simulate those used in commercial practice. The methods differ for each commodity and will be discussed in the appropriate commodity sections. Because the moisture content of stored commodities usually must be maintained within critical limits to prevent spoilage, the minimum spray volume consistent with thorough coverage should be used in these larger tests.

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Evaluation: -- Due to the larger size of the test units, efficacy must be evaluated by different means in pilot scale tests than in laboratory tests. A useful technique, particularly for dried fruits, nuts and tobacco leaves, is simply to evaluate damage at suitable intervals throughout the storage period. Random samples or entire test units can be evaluated. Generally, the examination of entire test units will preclude their reuse, making necessary the treatment of sufficient numbers of units to provide replicates for examination at each time interval. Sampling also poses a problem in that the insect infestations and resulting damage probably will be localized within the test commodity units, and care must be exercised to assure that the samples drawn are representative of the entire test unit and that the sampling does not affect subsequent infestation of the test unit. The level of damage may be assessed by applying industry grading standards or other quantitative measurements such as percentage of nut meats or leaves damaged. In making these assessments, insect counts should also be made.

An indirect method of evaluating efficacy involves removing a representative sample or group of samples from each test unit at appropriate intervals. The samples can then be examined for infestation and placed in containers in the laboratory and assayed for toxicity by infesting with eggs or neonate larvae. This method will not in all cases conclusively demonstrate efficacy, and must be supplemented by examination of representative samples of the commodity for damage and infestation at the end of the storage period.

A particularly useful method for detecting infestations in commodities stored in individual bins or other containers involves the placement of spools (ca. 2 cm wide x 4 cm in diameter) or strips (ca. 2 cm wide) of corrugated paper on the commodity surface. Mature larvae of most species of Lepidoptera that infest stored products will migrate to the surface for pupation and many individuals will seek out and pupate in this corrugated paper. The corrugated paper should be replaced weekly and returned to the laboratory for examination. The paper can be separated for counting the pupae, or it can be held in jars until adults emerge. The latter is preferred if data are desired for more than one species, as the adults are more easily identified. Other evidence of pupation at the commodity surface such as webbing of particles or kernels together to form cocoons may also be noted and quantitated. This means of efficacy evaluation should be supplemented by damage assessment and laboratory assays of the toxicity of periodically removed samples, and by damage assessment upon termination of the test.

References

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LaHue, D. W. 1971. Controlling the Indian meal moth in shelled corn with dichlorvos PVC resin strips. USDA, ARS. *Research Report No. 51-42.* 9 pp.

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McGaughey, W. H. 1970. Evaluation of dichlorvos for insect control in stored rough rice. *J. Econ. Entomol.* 63: 1867-1970.

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Commercial-Scale Testing

Purpose and Scope: -- Commercial-scale tests are made only after the application rate has been narrowed to a single dose, or at most, 2 doses. These tests are generally considered demonstrations of efficacy, and because of the large quantities and high values of the commodities involved may or may not include control bins or storage units. Such demonstrations should be conducted in all geographical areas in which the commodities are normally stored. Standard industry handling and storage practices should be employed, and the tests should be conducted throughout an entire storage season. Repetition of the tests in a second year is desirable.

Design and Analysis: -- Because pest infestation pressures may be sporadic and artificial infestation undesirable, extensive replication may be required to obtain valid data. Statistical analysis of the results may be impractical as control units may not be possible. Evaluation of efficacy may be possible only by comparisons with earlier years, with remotely located bins or warehouses under differing storage and pest management conditions and practices, or with laboratory determinations of infestability made on samples of the commodity prior to treatment.

Application: -- The proposed commercial application technique should be used. The methods differ for each commodity and methods consistent with industry practice should be used. Because the moisture content of stored commodities usually must be maintained within critical limits to prevent spoilage, the minimum spray volume consistent with thorough coverage should be used.

Evaluation: -- Efficacy can be quantitatively measured in the same manner as outlined for pilot-scale tests.

References

Quinlan, J. K. 1972. Malathion surface sprays for controlling insect populations in stored shelled corn. *Proc. N. Central Branch Entomol. Soc. Amer.* 27: 65-67.

Quinlan, J. K. 1977. Surface and wall sprays of malathion for controlling insect populations in stored shelled corn. *J. Econ. Entomol.* 70: 335-336.

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Lepidoptera in Stored Grain

Bacillus thuringiensis and a granulosis virus have been evaluated for controlling infestations of the Indian meal moth and the almond moth in stored wheat and corn. Both insect species are serious pests of most raw and processed grains. Infestations are confined primarily to the exposed surface layers of the stored commodities. The larvae feed extensively and contaiminate the commodities with frass, insect parts, and silk. In severe infestations in grain the webbing may restrict air movement through the grain mass, causing moisture condensation and subsequent grain spoilage. Economic loss results from decreased quality of the commodity brought about by contamination, weight and nutrient loss, and grain spoilage, and from increased processing costs.

The test methods and procedures presented here should be applicable for all bulk stored grains, and may be applicable for other commodities such as inshell peanuts which are stored and handled in the same general manner as grain.

Laboratory Testing

Methods and procedures have recently been published for laboratory-scale evaluations of insect susceptibility, application techniques, formulation efficacy, compatibility with grain fumigants, stability in the grain storage environment, and interaction with nonsusceptible insect species. The methods used follow the general laboratory testing procedure discussed above. The references cited should be consulted for more specific details of the test methods.

Test Size: -- 10 g - 27 kg of grain held in Mason jars, cloth bags, or metal columns may be used.

Design and Analysis: -- 3-5 replications are normally used. Analysis of variance and multiple range tests are useful in interpreting the data.

Application: -- Aqueous suspensions or dusts can be poured onto the grain and incorporated by tumbling, shaking or rolling the grain in jars. Liquid volumes as high as 20 ml per kg of grain are acceptable in laboratory studies if the grain is held in open containers for several hours immediately following application to permit drying.

Evaluation: -- Hold the grain in filter paper or cloth-covered containers and infest with eggs or neonate larvae. Monitor adult emergence. Use 10-25 insects in samples smaller than 200 g, 50 insects in samples of 200 to 500 g and 100 or more insects in 500 g or larger samples.

Pilot-Scale Testing

Tests beyond laboratory-scale have not been reported, however pilot-scale tests to evaluate the efficacy of *Bacillus thuringiensis* for Indian meal moth and almond moth control on stored wheat and corn are in progress. The procedures outlined here are being used in those tests.

Test Size: -- The bin size to be used in pilot-scale testing on grain depends upon the insecticide application procedure. Tests to evaluate treatment of the entire grain bulk can be accomplished in 3-5 bushel lots held in garbage pail-size containers. Tests to evaluate surface-layer treatments for moth control should be made in considerably larger lots of grain to accommodate the surface-layer infestation behavior of the moths. The minimum bin size for the latter is unknown, but bins of ca. 50 bushels should be appropriate.

Design and Analysis: -- The treated and untreated bins should be replicated at least 3 times. More replications are highly desirable. A randomized block bin arrangement may be used. Infestation and damage in the treated and untreated bins should be compared using standard statistical procedures.

Application: -- The pathogen can be applied to the grain as it is being mixed in a cement mixer or other type of rotating drum, as it is borne on a belt-type conveyor, or as it is being moved by auger from one bin to another. Thorough mixing with the grain may be necessary. In tests of surface-layer treatments, the insecticide can be applied in the same manner to the portion of grain to be treated prior to or as that portion is being placed in the bins. Or the pathogen may be applied to the grain surface after all the grain is in the bin. When this method is used the dose should be divided among 2 or more applications and the grain should be thoroughly mixed to the appropriate depth using a scoop or other implement following all but the final application. Conventional spraying equipment can be used to apply liquids, and liquids or powders can be sprinkled onto the grain if the subsequent mixing is sufficient to assure uniform coverage of the grain.

Evaluation: -- The bins should be individually infested with eggs or pupae of the insect species to be tested at intervals throughout that part of the storage period when the temperature will permit insect development. Efficacy of moth preventive treatments should be monitored using spools of corrugated paper as discussed in the general methods. Other evidence of infestation such as cocoons, live insects and webbing should be recorded at suitable intervals. Additionally small (ca. 300 g) samples of grain should be removed periodically from the treated zone of grain in each bin and examined for infestation. The samples can then be frozen to eliminate any infestation, placed in Mason jars in the laboratory, and infested with eggs of the insect species being tested. Reduction of adult emergence will be a measure of efficacy.

If tests are being made against species that infest the entire grain mass, samples should be removed at suitable intervals from all parts of the mass and examined for infestation.

Commercial-Scale Testing

Full-scale tests should be made to confirm the results obtained in pilot scale testing. None have been made in grain. However, they should conform to the general test methods discussed above.

References

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Kinsinger, R. A., and W. H. McGaughey. 1976. Stability of *Bacillus thuringiensis* and a granulosis virus of *Plodia interpunctella* on stored wheat. *J. Econ. Entomol.* 69: 149-154.

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Lepidoptera in Stored Tobacco

Bacillus thuringiensis has been evaluated for controlling the tobacco moth in tobacco stored in farm warehouses. This insect is a pest of numerous stored agricultural products, but in flue-cured tobacco it feeds and overwinters in stored or waste tobacco in warehouses. Newly hatched larvae feed on the lamina of the stored leaves near the stem end and feeding progresses toward the leaf tips as the larvae mature. The presence of small larvae can be noted only upon extremely close examination. Economic damage is caused by half-grown and larger larvae which may or may not be present at the time of examination. Heavy larval feeding may result in the presence of abundant silk and frass, and in 10-20% loss of leaf lamina. Such infestations have little effect on the weight of tobacco, however the effect on tobacco grades is drastic. The presence of foreign matter (silk, frass or insects) or insect damage reduces the assigned grade of the tobacco to "no grade" and results in significant economic loss.

Laboratory Testing

The susceptibility of the tobacco moth to candidate insect pathogens may be determined in small-scale laboratory tests by incorporating the pathogen into corn meal, fortified larval diet or other cereal products that may be suitable for larval development. Extrapolation of doses from these tests may be useful in selecting doses for pilot testing on tobacco leaves.

Pilot-Scale Testing

Test Size: -- Tobacco is normally stored in warehouses in bundles or sheets of 68-91 kg of leaves. In the initial testing when several doses are used, smaller bundles containing approximately 180 leaves and weighing 1.3-1.6 kg may be used. These smaller units, assembled in the same manner as the larger commercial size bundles, permit more extensive replication while conserving commodity.

Design and Analysis: -- Treated bundles should be stored in completely randomized block arrangement. 3-5 replications should be used. Standard statistical procedures are useful for comparing the levels of damage in the treated and untreated bundles.

Application: -- The tobacco leaves are sprayed with water as they are assembled in bundles to raise the moisture content and prevent loss in handling. Candidate insect pathogens may be incorporated in this water and applied directly to the individual leaves as the bundles are assembled.

Evaluation: -- After treatment the bundles (including replicated control bundles) should be stored in a heavily infested warehouse or room. Insect infestation in the warehouse should be supplemented if necessary to assure heavy infestation pressures. Other means of pest control which might affect the maintenance of a heavy infestation should be avoided. Exclusive use of a warehouse would be required as marketable tobacco could not be stored there. A sufficient number of bundles should be treated to permit removal of replicated bundles of each dose and the controls at various time intervals throughout the normal storage period.

Upon removal from storage the individual leaves in each bundle should be examined for damage and the presence of insects, frass or silk. The degree of damage may be assessed by applying industry grading standards or any other quantitative means of assessment. Other factors that have been used are percentage of leaves damaged to any degree, percentage of leaves damaged 5% or more, percentage loss per damaged leaf, percentage of leaf area loss per bundle, weight loss per bundle and market value loss. In general, however, noting the presence or absence of damage or foreign matter is sufficient, given current grading and marketing standards. If leaf position on the growing plant or other quality factors affect the damage that may result in storage, these factors should be evaluated in these small bundle tests.

Commercial-Scale Testing

Test Size: -- Data from the small bundle tests should be adequate to permit selection of a single dose for testing on a commercial scale using standard-size leaf bundles.

Design and Analysis: -- If possible, untreated bundles should be stored along with the treated bundles to serve as controls. However, pest management practices which should be in use in commercial warehouses and treatement of most of the bundles stored there may prevent all but sporadic infestation of the control bundles. The high value of the commodity may preclude testing in artificially infested warehouses. Statistical analysis may be impractical.

Application: -- The pathogen should be applied using the proposed commercial practice. Incorporation with water sprayed onto leaves as they are assembled in bundles would be most desirable.

Evaluation: -- At intervals throughout the storage period random bundles should be removed from storage and the leaves in approximately 1.3 kg samples from the top, middle and bottom of representative bundles examined for damage or foreign matter in the same manner as described for pilot-scale tests.

References

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Mistric, W. J., Jr. 1977. Tobacco moth control on farm-stored tobacco. *Proc. Entomol. Soc., 27th Tobacco Workers Conf.* January 1977. Atlanta, Georgia. pp. 4-6.

Lepidoptera in Dried Fruits and Nuts

Storage Infestation

A granulosis virus of the Indian meal moth has been evaluated to a limited extent for controlling Indian meal moth infestations in stored inshell almonds, walnuts and peanuts, and in field run and processed (packaged) raisins. The Indian meal moth is a severe storage pest of each of these commodities. Economic damage results from larval feeding. While weight loss of the commodities may be minimal, the most serious losses are in appearance and quality, resulting from larval feeding and from contamination by frass, silk and insects. Market value may be lowered or destroyed, and processing costs may be increased as a result of the need to clean and sort the infested commodities.

Laboratory Testing

The susceptibility of the Indian meal moth larvae and larvae of certain other lepidopterous pests of dried fruits and nuts to candidate insect pathogens may be determined in small-scale laboratory tests by incorporating the pathogen into cereal product diets or other suitable larval foods. Extrapolation of doses from these tests may be useful in selecting doses for testing on the commodity.

Test Size: -- Initial testing of multiple doses on a commodity can be accomplished by treating 100-1000 g samples. Tests of this size should provide adequate data to narrow the dose range to 2 or 3 levels in subsequent tests.

Application: -- Application may be made by spraying, dusting or dipping.

Evaluation: -- The treated commodity can be held in Mason jars or other suitable containers and infested with eggs or neonate larvae. Larval survival or adult emergence can be used as measures of efficacy.

Pilot-Scale Testing

Test Size: -- 6-12 kg of nuts per test unit have been used. However, larger quantities have been treated using standard industry equipment and subdivided into smaller samples for evaluation of efficacy.

Application: -- The pathogen may be applied by spraying the commodity as it is borne on a belt or other type of mechanical conveyor.

Evaluation: -- The commodities treated with each dose can be divided into replicate samples and held in jars or cloth-covered containers such as garbage pails or barrels. The containers can then be held in actual or simulated storage environments and periodically infested with eggs or neonate larvae. The emergence of moths from the containers should be monitored to evaluate efficacy. If the containers are large enough, small samples of the commodity can be withdrawn periodically throughout the storage period and subjected to quality determination, including taste panel tests, either through the application of industry grading procedures or other reliable means.

Commercial-Scale Testing

While no commercial-scale tests have been reported, these tests should conform to the general procedures discussed above.

References

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Field Investigation

Large-scale tests have been made of the efficacy of *Bacillus thuringiensis* for controlling the navel orangeworm in almonds. Infestation of almonds by this pest occurs in the field at about the time of hull crack. Larvae are transported into storage with the harvested nuts where they continue to feed on the nut meats until mature. Reproduction may occur in stored nuts. The methods used appear to be appropriate, but the effectiveness of the treatments is too inconclusive to permit a valid assessment of those methods.

References

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Lepidoptera in Processed Cereal Products

Processed cereal products are subject to infestation by a number of species of Lepidoptera and Coleoptera. The damage and economic consequences of these infestations closely parallel those occurring in whole grains. Species of both orders may cause contamination, and species of Lepidoptera may cause webbing in the products. Either results in economic loss through the costs of and losses through cleaning of the infested commodity.

Studies with insect pathogens have been made only on a very limited laboratory scale. The insects studied have included the Indian meal moth, almond moth and others, but the Mediterranean flour moth has been the predominant species. Because only limited tests have been made, detailed methodology is unavailable. The General Methods section and the references cited should be useful in developing appropriate procedures.

Bacillus thuringiensis powders have been mixed with small samples (generally less than 1000 g) of wheat flour by tumbling in jars. Eggs or neonate larvae were introduced and larval survival or adult emergence were monitored to determine efficacy. In one test, *Bacillus thuringiensis* was dusted and sprayed into small sheds containing residues of flour. These sheds were designed and specially constructed to simulate a flour storage warehouse that would contain deposits of spilled flour in cracks and corners and on structural components of the building. The sheds were subsequently infested with larvae of the Mediterranean flour moth. Efficacy was monitored through 2 generations of the insects by comparing insect counts and the percentage by weight of the flour that was webbed in the treated and untreated sheds.

References

Burges, H. D. 1964. Insect pathogens and microbial control of insects in stored products. I. Test with *Bacillus thuringiensis* Berliner against moths. *Entomophaga Mem. Hors. Ser. No. 2.* pp. 323-327.

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Other Stored Commodities

Beeswax and Beecomb

Specialized techniques are necessary for evaluating microbial agents for controlling pests of beeswax and beecomb in storage or in beehives. References are cited for studies that have been reported for controlling the greater wax moth and the lesser wax moth using *Bacillus thuringiensis*. These studies cite methods for determining larval susceptibility using fortified larval diets and for determining the efficacy of pathogens incorporated in foundation comb wax.

References

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EXHIBIT 1

ASSESSMENT OF PLANT COVERAGE AND FIELD PERSISTANCE OF ENTOMOGENOUS BACTERIA AND BACULOVIRUSES APPLIED FOR PEST CONTROL

Several approaches have been utilized to determine coverage and field persistence of pathogens including spore assay, bioassay, tracers, scanning electron microscope and collection of organisms to assess impact of treatment.

Spore Assay (Entomogenous Bacteria)

Parts to be examined (leaves, fruit, bark, stems) are removed from treated trees and placed in separate vials without preservative. In the laboratory, a known area is removed from each sample (e.g., 8 mm diameter disc from the center of each leaf with a sterile cork-borer). These are transferred to stoppered test tubes containing a measured amount of sterile water (sufficient to cover) and about ten 3 mm diameter glass beads. The tubes are shaken vigorously (10 minutes for leaf discs), and samples removed for serial dilution and counting. The method of Miles and Misra (1938) using Difco^R brain-heart infusion agar plates is employed. Generally, seven replicate counts are made of each suspension (Pinnock, et al. 1971, 1975; Brand et al. 1975). This method is useful to assess the coverage of products containing *Bacillus thuringiensis*. Furthermore, this technique can be employed to assess coverage of non-bacterial control agents (e.g., baculoviruses, chemicals) by adding a known amount of a *B. thuringiensis* product and following the procedures for spore assay (Sorensen 1977).

Bioassay

One way to conduct bioassays to determine coverage is to place all leaves comprising one sample in an 8-oz Dixie^R cup, add ten neonate larvae and cover with a tightly fitted plastic lid. The larvae are allowed to feed on the leaves for 48 hours. They are then transferred to individual 7-dram plastic vials containing formaldehyde-free rearing diet. The larvae are observed at frequent intervals and mortality recorded (Gard 1975). Other bioassay methods are available (see General Reference section).

Tracer

Coverage may also be determined by the fluorescent particle spray droplet tracer method (Himel 1969). Calcofluor or other fluorescent paints can be added to the spray mixture (Davidson and Pinnock 1971). A simple method is to place white cards in the area to be treated and add a dye to the spray mixture. The spray droplets will be visible on the card and provide a basis for determining spray droplet size, density and distribution.

Scanning Electron Microscope (SEM)

Many types of SEM and various procedures for using them are available. An example given here is for use with a Cambridge Mark II SEM. Treated plant parts are affixed to aluminum foil with silver conducting paint. Specimens are placed in a vacuum evaporator and coated with 50 A^{o} thickness of aluminum. The aluminum foil is attached to a specimen holder designed for the SEM, using a silver conducting paint as glue. The specimens are placed in the SEM and scanned. Number of particles visible for a known area can be determined.

Collecting Organisms to Assess Impact of Treatments

Available stages of target and non-target organisms are collected and held individually under conditions favorable for survival and development. The organisms are observed frequently, and all dead individuals are diagnosed to determine cause of death.

For codling moth on apple, for example, this can be done by collecting a specific quantity of visibly infested fruit at predetermined intervals following applications (e.g., ten fruit per plot at 0, 1, 2, 4, 8, 16 days following treatment) (Falcon et al. 1968).

References

Brand, R. J., D. E. Pinnock, K. L. Jackson, and J. E. Milstead. 1975. Methods for assessing field persistence of *Bacillus thuringiensis* spores.

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EXHIBIT 2

SUGGESTED PROTOCOL FOR EVALUATION OF MICROBIAL PATHOGENS FOR CONTROL OF SELECTED INSECTS ON SOYBEANS

Target Insects:

Soybean Looper, *Pseudoplusia includens*

Cabbage Looper, *Trichoplusia ni*

Corn Earworm, *Heliothis zea*

Microbial Pathogens:

Pseudoplusia NPV

Trichoplusia NPV

Heliothis NPV

Bacillus thuringiensis var. *kurstaki*

Plot Size:

0.1 Acre (minimum)

Replications:

4 (minimum)

Treatments and Rates:

- *Pseudoplusia* NPV for soybean looper control
    (1) 20, 40 and 8 L.E.*/A and untreated check

L.E. = Larval Equivalent = 6 x 10^9 Polyhedral Inclusion Bodies

- *Trichoplusia* NPV for cabbage looper control
  (1) 10, 20 and 40 L.E./A and untreated check
- *Heliothis* NPV for corn earworm control
  (1) 20, 40 and 80 L.E./A and untreated check
- *B. Thuringiensis* var. *kurstaki* for soybean looper control
  (1) 2×10^9, 4×10^9 and 8×10^9 I.U./A and untreated check
- Combinations of NPV's for control of mixed target insect populations
  (1) When mixed populations of the target insects occur, the NPV of each species should be tested independently and in combination at the rates above, e.g., with mixed soybean looper and cabbage looper populations, treatments would be as follows: *Pseudoplusia* NPV - 20, 40 and 80 L.E./A; *Trichoplusia* NPV - 10, 20 and 40 L.E./A; *P.* NPV - L.E./A + *T.* NPV - 10 L.E./A; *P.* NPV - 40 L.E./A + *T.* NPV - 20 L.E./A; *P.* NPV - 80 L.E./A + *T.* NPV - 40 L.E./A and untreated check.

  Time and labor permitting, all possible rate combinations would be desirable.

Formulations:

Pseudoplusia NPV may be provided by University of Arkansas. (See attached memorandum for others).

Application Equipment:

Boom-type sprayer

Initial Application:

To be determined by populations in each area — a minimum of 10-20,000 larvae/A suggested. Applications should be made before larvae reach the third stadium.

Spray Interval

With uneven aged populations, applications should be continued at 4-6 day intervals until a majority of the population reaches the fourth stadium.

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